SelectHealth Medical Policies

Laboratory Utilization Policies, Part 2

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Laboratory Utilization Policies (Part 2), Continued

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See Laboratory Utilization Policies (Part 1) for the following:

Immunohistochemistry

Policy #: AHS –P2018	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Immunohistochemistry (IHC) is a very sensitive and specific staining technique that uses anatomical, biochemical, and immunological methods to identify cells, tissues, and organisms by the interaction of target antigens with highly specific monoclonal antibodies and visualization though the use of a biochemical tag or label (Fitzgibbons et al., 2014).

II. Related Policies

Policy Number	Policy Title

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Code 88342 should be used for the first single antibody procedure and is reimbursed at one unit per specimen, up to four specimens, per date of service.
2. Code 88341 should be used for each additional single antibody per specimen and is reimbursed up to a maximum of 13 units per date of service.
3. Code 88344 should be used for each multiplex antibody per specimen, up to six specimens, per date of service.

IV. Scientific Background

Immunohistochemistry (IHC) is used to identify certain components of tissues or cells (aka immunocytochemistry) via use of specific antibodies that can be visualized through a staining technique. The premise behind IHC is that distinct tissues and cells contain a unique set of antigens that allows them to be identified and differentiated. The selection of antibodies used for the evaluation of a specimen varies by the source of the specimen, the question to be answered, and the pathologist performing the test.

Importantly, an entirely sensitive and specific IHC marker rarely exists, and therefore, determinations are typically based on a pattern of positive and negative stains for a panel of several antibodies. The four most common IHC staining patterns include nuclear staining, cytoplasmic staining, membrane staining, and extracellular staining (Tuffaha et al., 2018). A single IHC marker approach (other than for pathogens such as cytomegalovirus or BK virus) is strongly discouraged since aberrant expression of a highly specific IHC marker can rarely occur. However, aberrant expression of the entire panel of highly specific IHC markers is nearly statistically impossible (Lin & Chen, 2014).

Multiplex immunohistochemistry (mIHC) is a particular IHC technique that allows multiple targets in a single tissue to be detected simultaneously; this approach is able to characterize "the tumor microenvironment including vascular architecture and hypoxia, cellular proliferation, cell death as well as drug distribution" (Kalra & Baker, 2017). Hence, mIHC can assist in the development of parameter tumor maps. Other researchers have utilized mIHC for its novel ability to provide quantitative data on different types of tumor-infiltrating immune cells within a single tissue; this may improve cancer patient immunotherapy stratification (Hofman et al., 2019).

Clinical Utility and Validity

IHC can be used for a variety of purposes including: differentiation of benign from malignant tissue, differentiation among several types of cancer, selection of therapy, identification of the origin of a metastatic cancer, and identification of infectious organisms (Shah et al., 2012). IHC has many uses in the realm of tumor identification, and it has even been clinically used to pinpoint various breast cancer-specific markers, such as progesterone and estrogen receptors, gross cystic duct fluid protein, and mammaglobin (Hainsworth & Greco, 2022). Further, overexpression of the *HER2* oncogene, a predicative breast cancer biomarker, is often identified via IHC (Yamauchi & Hayes, 2022). In regards to tumor identification, a specific type of IHC, known as pan-Trk IHC, has been shown to positively identify inflammatory myofibroblastic tumors with a nuclear and cytoplasmic staining pattern that may assist in targeted therapy (Yamamoto et al., 2019).

Antibodies for use in IHC are available as single antibody reagents or in mixtures of a combination of antibodies. More than 200 diagnostic antibodies are generally available in a large clinical IHC laboratory, and hundreds of antibodies are usually available in research laboratories. The list of new antibodies is growing rapidly with the discovery of new biomarkers by molecular methodologies (Lizotte et al., 2016). Several studies have shown that a relatively low number of antibodies are capable of accurately diagnosing specific cancers and identifying the primary source of a metastasis (Le Stang et al., 2019; Lizotte et al., 2016; Prok & Prayson, 2006).

Immunohistochemistry, continued

Common markers to identify tumor origin (Lin & Chen, 2014):

Primary Site	Markers
Lung adenocarcinoma	TTF1, napsin A
Breast carcinoma	GATA3, ER, GCDFP15
Urothelial carcinoma	GATA3, UPII, S100P, CK903, p63
Squamous cell carcinoma	p40, CK5/6
RCC, clear cell type	PAX8, RCCma, pVHL, KIM-1
Papillary RCC	P504S, RCCma, pVHL, PAX8, KIM-1
Translocational RCC	TFE3
Hepatocellular carcinoma	Arginase-1, glypican-3, HepPar-1
Adrenal cortical neoplasm	Mart-1, inhibin-a, calretinin, SF-1
Melanoma	S100, Mart-1, HMB-45, MiTF, SOX10
Merkel cell carcinoma	CK20 (perinuclear dot staining), MCPyV
Mesothelial origin	Calretinin, WT1, D2-40, CK5/6, mesothelin
Neuroendocrine origin	Chromogranin, synaptophysin, CD56
Upper GI tract	CDH17, CDX2, CK20
Lower GI tract	CDH17, SATB2, CDX2, CK20
Intrahepatic cholangiocarcinoma	pVHL, CAIX
Pancreas, acinar cell carcinoma	Glypican-3, antitrypsin
Pancreas, ductal adenocarcinoma	MUC5AC, CK17, Maspin, S100P, IMP3
Pancreas, neuroendocrine tumor	PR, PAX8, PDX1, CDH17, islet-1
Pancreas, solid pseudopapillary tumor	Nuclear b-catenin, loss of Ecadherin, PR, CD10, vimentin
Prostate, adenocarcinoma	PSA, NKX3.1, PSAP, ERG
Ovarian serous carcinoma	PAX8, ER, WT1
Ovarian clear cell carcinoma	pVHL, HNF-1b, KIM-1, PAX8
Endometrial stromal sarcoma	CD10, ER
Endometrial adenocarcinoma	PAX8/PAX2, ER, vimentin
Endocervical adenocarcinoma	PAX8, p16, CEA, HPV in situ hybridization, loss of PAX2
Thyroid follicular cell origin	TTF1, PAX8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF1, CEA

Immunohistochemistry, continued

Hyalinizing trabecular adenoma of the thyroid	MIB-1 (unique membranous staining pattern)
Salivary duct carcinoma	GATA3, AR, GCDFP-15, HER2/neu
Thymic origin	PAX8, p63, CD5
Seminoma	SALL4, OCT4, CD117, D2-40
Yolk sac tumor	SALL4, glypican-3, AFP
Embryonal carcinoma	SALL4, OCT4, NANOG, CD30
Choriocarcinoma	b-HCG, CD10, SALL4
Sex cord–stromal tumors	SF-1, inhibin-a, calretinin, FOXL2
Vascular tumor	ERG, CD31, CD34, Fli-1
Synovial sarcoma	TLE1, cytokeratin
Chordoma	Cytokeratin, S100
Desmoplastic small round cell tumor	Cytokeratin, CD99, desmin, WT1 (N-terminus)
Alveolar soft part sarcoma	TFE3
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Smooth muscle tumor	SMA, MSA, desmin, calponin
Ewing sarcoma/PNET	NKX2.2, CD99, Fli-1
Myxoid and round cell liposarcoma	NY-ESO-1
Low-grade fibromyxoid sarcoma	MUC4
Epithelioid sarcoma	Loss of INI1, CD34, CK
Atypical lipomatous tumor	MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4
Histiocytosis X	CD1a, S100
Angiomyolipoma	HMB-45, SMA
Gastrointestinal stromal tumor	CD117, DOG1
Solitary fibrous tumor	CD34, Bcl2, CD99
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers; may lose INI1
Myeloid sarcoma	CD43, CD34, MPO
Follicular dendritic cell tumor	CD21, CD35
Mast cell tumor	CD117, tryptase

V. Guidelines and Recommendations

Guidelines are lacking regarding the selection and number of antibodies that should be used for most immunohistochemistry evaluations. However, IHC is broadly used for conditions such as cancers, which are mentioned across many different societies. The below section is not a comprehensive list of guidance for immunohistochemistry.

College of American Pathologists (CAP)

CAP has published several reviews in Archives of Pathology & Laboratory Medicine that detail the quality control measures for IHC; further, CAP has also published more than 100 small IHC panels to address the frequently asked questions in diagnosis and differential diagnosis of specific entities. These diagnostic panels are based on literature, IHC data, and personal experience. A single IHC marker approach (other than for pathogens such as cytomegalovirus or BK virus) is strongly discouraged since aberrant expression of a highly specific IHC marker can rarely occur. However, aberrant expression of the entire panel of highly specific IHC markers is nearly statistically impossible (Lin & Chen, 2014; Lin & Liu, 2014).

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP)

ASCO and CAP currently recommend that "all newly diagnosed patients with breast cancer must have a HER2 test performed" (Wolff et al., 2013). Also, for those who develop metastatic disease, a HER2 test must be done on tissue from the metastatic site, if available. In less common HER2 breast cancer patterns, as observed in approximately 5% of cases by dual-probe in situ hybridization (ISH) assays, new recommendations have been made to make a final determination of positive or negative HER2 tissue. This new "diagnostic approach includes more rigorous interpretation criteria for ISH and requires concomitant IHC review for dual-probe ISH groups… to arrive at the most accurate HER2 status designation (positive or negative) based on combined interpretation of the ISH and IHC assays;" further, "The Expert Panel recommends that laboratories using single-probe ISH assays include concomitant IHC review as part of the interpretation of all single-probe ISH assay results" (Wolff et al., 2018).

The National Cancer Coalition Network

The NCCN has made numerous recommendations for use of IHC to diagnose and manage various types of cancer. Cancers with clinically useful IHC applications include breast, cervical, various leukemias, and colorectal cancer.

The NCCN states that the determination of estrogen receptor, progesterone receptor, and HER2 status for breast cancer is recommended and may be determined by IHC (NCCN, 2022a). Specifically, the NCCN guidelines state "consistent with the ASCO/CAP guidelines, the NCCN panel considers either IHC or ISH with either a single or dual probe as an acceptable method for making an initial determination of HER2 tumor status. PR testing by IHC on invasive cancers can aid in the prognostic classification of cancers and serve as a control for possible false negative ER results. Patients with ER-negative, PR-positive cancers may be considered for endocrine therapies, but the data on this group are noted to be limited" (NCCN, 2022a). Further, the NCCN recommendations concerning Lynch Syndrome (LS) state, "The panel recommends tumor testing with IHC and/or MSI be used as the primary approach for pathology-lab-based universal screening" (NCCN, 2022b). More recently, the NCCN has made additional recommendations to individuals diagnosed with any type of hereditary colorectal cancer (CRC) syndrome; these recommendations state that "all individuals newly diagnosed with CRC have either MSI or immunohistochemistry (IHC) testing for absence of 1 of the 4 DNA MMR proteins" (NCCN, 2022b).

VI. Applicable State and Federal Regulations

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Recently, four clinical IHC biomarker assays (PTEN, RB, MLH1, and MSH2) have been validated for use as biomarkers in a nationwide clinical trial; these assays were then approved by the FDA as laboratory-developed tests to assist in the treatment selection of patients in clinical trials (Khoury et al., 2018). This shows that IHC assays are currently being utilized with molecular tests to assist in therapeutic decisions.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure
88342	Immunohistochemistry or immunocytochemistry, per spec; initial single antibody stain
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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Immunohistochemistry, continued

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IX. Revision History

Revision Date	Summary of Changes

Immunopharmacologic Monitoring of Therapeutic Serum Antibodies

Policy #: AHS - G2105	**Prior Policy Name & Number (as applicable):** • AHS - G2105 - Immunopharmacologic Monitoring of Infliximab, Adalimumab and Other Therapeutic Serum Antibodies • AHS - G2105 - Measurement of Serum Antibodies to Infliximab and Adalimumab • AHS - G2105 - Immunopharmacologic Monitoring of Infliximab and Adalimumab
Implementation Date: 9/15/21	**Date of Last Revision:** 4/28/22, 2/20/23 (See Section IX)

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I. Policy Description

Immunopharmacologic monitoring of circulating drug and anti-drug antibody levels has been proposed to manage loss of response due to the development of anti-drug antibodies, which may promote adverse effects and diminish drug efficacy (Bendtzen, 2017; Tighe & McNamara, 2017).

Targeted inhibitors of tumor necrosis factor-alpha (TNF), including, but not limited to, infliximab, adalimumab, etanercept, and golimumab, are widely used in the treatment of a number of inflammatory conditions, including rheumatoid arthritis (RA), spondyloarthritis, inflammatory bowel disease, and psoriasis (Bendtzen, 2021).

II. Related Policies

Policy Number	Policy Title
AHS-G2098	Immune Cell Function Assay
AHS-G2127	Vectra DA Blood Test for Rheumatoid Arthritis
AHS-G2155	General Inflammation Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1) In individuals with inflammatory bowel disease, drug and/or antibody concentration testing for anti-tumor necrosis factor (anti-TNF) therapies **MEETS COVERAGE CRITERIA** in the following situations:
   a) At the end of induction for all anti-TNFs
   b) At least once during maintenance therapy
   c) At the end of induction in primary non-responders
   d) In patients with confirmed secondary loss of response

2) In individuals with inflammatory bowel disease, drug and/or antibody concentration testing for vedolizumab or ustekinumab therapies **MEETS COVERAGE CRITERIA** in the following situations:
   a) In non-responders at the end of induction
   b) In patients with confirmed secondary loss of response

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3) For individuals without inflammatory bowel disease (e.g., spondyloarthritis, rheumatoid arthritis, psoriatic arthritis, and psoriasis), drug and/or antibody concentration testing for anti-TNF therapies **DOES NOT MEET COVERAGE CRITERIA.**

4) Measurement of the serum drug levels and/or measurement of the antibodies to the following drugs for any other reason, either alone or as a combination test, in an outpatient setting **DOES NOT MEET COVERAGE CRITERIA**:
   a) adalimumab
   b) certolizumab
   c) etanercept
   d) golimumab

e) infliximab
f) infliximab-dyyb
g) infliximab-abda
h) rituximab
i) ustekinumab
j) vedolizumab

IV. Scientific Background

Tumor necrosis factor (TNF) inhibitors competitively inhibit the binding of TNF to its receptors to reduce inflammation and stop disease progression (Lis et al., 2014). They are used for treatment of inflammatory conditions, such as rheumatoid arthritis (RA), psoriatic arthritis, juvenile arthritis, inflammatory bowel disease (Crohn's and ulcerative colitis), and ankylosing spondylitis (Bendtzen, 2019b; Lis et al., 2014). Five primary biologic TNF inhibitors are used for inflammatory diseases; infliximab, adalimumab, certolizumab pegol, golimumab, and etanercept. However, these inhibitors may lead to the formation of auto-drug antibodies, which may hinder treatment and cause other adverse effects, such as allergic reactions (Bendtzen, 2017).

TNF inhibitors are a subset of biologic disease-modifying antirheumatic drugs (bDMARDs), which "improve symptoms and reduce structural damage of joints, the gastrointestinal tract, and other affected organs." However, patients oftentimes do not respond to treatment, with upwards of 50% of patients attaining "secondary failure," or inadequate disease control. Important contributors to the secondary failure include anti-drug antibodies and low drug concentrations, which may then contribute to antidrug antibody formation. Generally, the approach to prescribing bDMARDs, such as infliximab, is to adjust or switch "only when there is clinical evidence that remission or low disease activity is not achieved or maintained, which may occur months after treatment initiation." Sometimes, drugs like methotrexate may be prescribed along with the bDMARDs to prevent antidrug antibody development. Guidelines recommending therapeutic drug monitoring (TDM) also vary by inflammatory disease – for example, it is recommended for inflammatory bowel disease (IBD) but not rheumatoid arthritis (RA). To prevent the drawbacks of using bDMARDs from accumulating further, proactive TDM is best supported, but it does not come without barriers like additional personnel needed for constant monitoring, and a dearth of understanding of how other bDMARDs are affected similarly or differently (Wallace & Sparks, 2021).

Proprietary Testing

To optimize dosing of TNF inhibitors, therapeutic drug monitoring (TDM) of both these drugs as well as anti-drug antibodies has been proposed. This dual monitoring is thought to help clinicians manage drug regimens for these patients, such as adjusting the dose or changing the drug entirely. Identifying the presence and concentration of these drugs and auto-drug antibodies may help avoid nonresponse to treatment. Most assays for the assessment of serum antibodies will also report the drug concentration (Lichtenstein, 2021). For example, HalioDx Inc. offers OptimAbs, which a set of assays for eight biologic agents (adalimumab, certolizumab pegol, golimumab, infliximab, infliximab-dyyb, infliximab-abda, ustekinumab, and vedolizumab). These assays are intended to allow providers to monitor, manage

response, and optimize dose (Theradiag, 2018). Prometheus Anser also offers a series of assays for assessment of these anti-drug antibodies, with assessments for four biologics (adalimumab, infliximab, ustekinumab, and vedolizumab). They also measure the levels of antibodies against the drug in question (Anser, 2019). LabCorp offers eight assays for 10 biologics (adalimumab, certolizumab, etanercept, golimumab, infliximab, infliximab- dyyb, infliximab-abda, rituximab, ustekinumab, and vedolizumab) encompassed in one portfolio called "DoseASSURE" (LabCorp, 2019).

Clinical Utility and Validity

S. L. Wang et al. (2012) developed and validated a non-radiolabeled homogeneous mobility shift assay (HMSA) to measure the antibodies-to-infliximab (ATI) and infliximab levels in serum samples. The assay was validated for both items, and the sample was compared to the traditional ELISA. Intra- and interassay precision rates for the ATI- and infliximab-HMSA were less than 4% and less than 15%, respectively, and less than 6% and less than 15%, respectively. The lower limit of quantitation of the ATI-HMSA was found to be 0.012 µg/mL in serum, and the HMSA correlated well with the ELISA for ATI levels.

S. L. Wang et al. (2013) developed and validated a non-radiolabeled HMSA to measure antibodies-to-adalimumab (ATA) and adalimumab levels in serum samples. Analytic validation of performance characteristics (calibration standards, assay limits, et al.) was performed for both the ATA- and adalimumab-HMSA. Because the elimination half-life of adalimumab (10-20 days) overlaps the dosing interval (every 2 weeks), ATA-positive sera to provide calibration standards were difficult to collect from human patients. The drug-free interval for antibody formation is small. Therefore, antisera from rabbits immunized with adalimumab were pooled to form calibration standards. Serial dilutions of these ATA calibration standards then generated a standard curve against which test samples were compared. With over 29 experimental runs, intra-assay precision and accuracy for the adalimumab-HMSA was <20% and <3%, respectively; interassay (run-to-run, analyst-to-analyst and instrument-to-instrument) precision and accuracy were less than 12% and less than 22%, respectively. For the ATA-HMSA, variance for intra-assay precision and accuracy were less than 3% and less than 13%, respectively; variance for interassay precision and accuracy were less than 9% and less than 18%, respectively (S. L. Wang et al., 2013). ELISA could not be used as a standard comparator due to competition from circulating drug.

Van Stappen et al. (2016) validated a rapid, lateral flow-based assay (LFA) for quantitative determination of infliximab and to assess thresholds associated with mucosal healing in patients with ulcerative colitis. They found that the LFA agreed well with the traditional enzyme-linked immunosorbent assay (ELISA) for quantification of infliximab with correlation coefficients of 0.95 during induction. A trough concentration (TC) of ≥2.1 µg/ml was associated with mucosal healing. They concluded, "With a time-to-result of 20 min, individual sample analysis and user-friendliness, the LFA outplays ELISA as a rapid, accurate tool to monitor infliximab concentrations" (Van Stappen et al., 2016).

Steenholdt et al. (2014) investigated "the cost-effectiveness of interventions defined by an algorithm designed to identify specific reasons for therapeutic failure." A total of 69 patients with secondary infliximab (IFX) failure were randomized to IFX dose intensification (n = 36) or interventions based on serum IFX and IFX antibody levels (n = 33). The researchers found that "Costs for intention-to-treat patients were substantially lower (34%) for those treated in accordance with the algorithm than by infliximab (IFX) dose intensification: €6038 vs €9178. However, disease control, as judged by response rates, was similar: 58% and 53%, respectively" (Steenholdt et al., 2014). They concluded that "treatment of secondary IFX failure using an algorithm based on combined IFX and IFX antibody measurements

significantly reduces average treatment costs per patient compared with routine IFX dose escalation and without any apparent negative effect on clinical efficacy" (Steenholdt et al., 2014).

Roblin et al. (2014) conducted a prospective study with 82 patients with inflammatory bowel disease (IBD) having a disease flare while being on ADA 40 mg every 2 weeks. All patients were primary responders to ADA therapy and were anti-tumor necrosis factor (TNF). ADA trough levels and antibodies against ADA (AAA) were measured. All patients were optimized with ADA 40 mg weekly. Four months later, in the absence of clinical remission, patients were treated with infliximab. The researchers concluded, "The presence of low ADA trough levels without AAA is strongly predictive of clinical response in 67% of cases after ADA optimization. Conversely, low ADA levels with detectable AAA are associated with ADA failure, and switching to IFX should be considered. ADA trough levels >4.9 µg/ml are associated with failure of two anti-TNF agents (ADA and IFX) in 90% of cases and switching to another drug class should be considered (Roblin et al., 2014)."

Mitchell et al. (2016) studied if infliximab (IFX) therapeutic drug monitoring (TDM) allows for objective decision making in patients with inflammatory bowel disease (IBD) and loss of response. A total of 71 patients with IBD that had IFX TDM were examined and their serum concentration of anti-drug antibodies were measured. Patients were grouped by TDM results: group 1, low IFX/high ADA; group 2, low IFX/low ADA; group 3, therapeutic IFX, and changes in management were examined due to groupings. Of the 71 patients, 37% underwent an "appropriate" change in therapy based on group. The

authors concluded, "A trend towards increased remission rates was associated with appropriate changes in management following TDM results. Many patients with therapeutic IFX concentrations did not undergo an appropriate change in management, potentially reflecting a lack of available out-of-class options at the time of TDM or due to uncertainty of the meaning of the reported therapeutic range (Mitchell et al., 2016)."

Barlow et al. (2016) evaluated the clinical utility of antibodies in relation to C-reactive protein concentrations. A total of 108 patients contributed 201 samples, and total anti-infliximab antibodies were measured in 164 samples. The authors found that median trough infliximab was 3.7 µg / mL, and 23% of the samples were ≤1 µg / mL. They also noted that "Serum C-reactive protein was found to be significantly higher where infliximab was ≤1 compared to >1 µg/mL," but no "strict" correlation was seen (Barlow et al., 2016). Approximately 85% of samples with positive anti-infliximab antibodies had infliximab ≤1 µg / mL, and the authors concluded, "Our findings support measurement of anti-infliximab antibodies only in the context of low infliximab concentrations <1 µg/mL. A higher therapeutic cut-off may be relevant in patients with negative antibodies. Further work is indicated to investigate the clinical significance of positive antibodies with therapeutic infliximab concentrations (Barlow et al., 2016)."

Moore et al. (2016) performed a systematic review and meta-analysis of studies that reported serum infliximab levels according to IBD outcomes. Twenty-two studies were examined, encompassing 3483 patients. Twelve studies reported IFX levels in a manner "suitable" for estimating the effect. The researchers found that "During maintenance therapy, patients in clinical remission had significantly higher mean trough IFX levels than patients not in remission: 3.1 µg/ml versus 0.9 µg/ml. The standardised mean difference in serum IFX levels between groups was 0.6 µg/ml. Patients with an IFX level > 2 µg/ml were more likely to be in clinical remission (risk ratio [RR]: 2.9), or achieve endoscopic remission [RR 3] than patients with levels < 2 µg/ml." The study concluded, "There is a significant difference between serum infliximab levels in patients with IBD in remission, compared with those who

relapse. A trough threshold during maintenance > 2 µg/ml is associated with a greater probability of clinical remission and mucosal healing (Moore et al., 2016)."

Wang et al. (2018) submitted an abstract to the 2018 Therapeutic Drug Management and Toxicology Division Abstract Competition conducted by the American Association for Clinical Chemistry (AACC) on July 30, 2018. This abstract focused on InformTx's assays for TDM, and the authors reviewed TDM results for six biologics: adalimumab (ADA), certolizumab (CER), golimumab (GOL), infliximab (INF), ustekinumab (UST), and vedolizumab (VED). A total of 18837 sera samples were analyzed with InformTx's assays, and patient responses were predicted based on drug and anti-drug antibody status (ADAbs). The need for drug optimization were assessed by comparing patient drug levels to recommended therapeutic drug levels and laboratory-defined higher ADAbs. The authors found that "64.1%, 30.2%, 83.9%, 60.4%, 25.2%, and 69.1% of the patients treated with ADA, CER, GOL, INF, UST, and VED, respectively, had drug level equal to or greater than the recommended therapeutic level and undetectable ADAbs." Approximately 4.5%-33% patients had a drug concentration above the recommended therapeutic level. In contrast, patients (31.0% in ADA, 57.0% in CER, 12.1% in GOL, 32.5% in INF, 74.4% in UST, and 30.6% in VED) had undetectable or suboptimal levels of drugs and undetectable or lower levels of ADAbs (Wang et al., 2018).

Fernandes et al. (2019) examined whether TDM can improve clinical outcomes in Crohn's disease (CD) and ulcerative colitis (UC) patients. A total of 205 patients were included in the study, and 56 patients were placed in a "proactive" regimen. This proactive regimen involved measuring infliximab (IFX) trough levels and antidrug antibodies before the fourth infusion and every two infusions. The regimen aimed to establish an IFX trough level of 3-7 ug/mL for CD patients and 5-10 ug/mL for UC patients. The control group was made of patients treated with IFX but without TDM. The authors found that treatment escalation was more common in the proactive TDM (pTDM) group (76.8% vs 25.5%), mucosal healing was more common, (73.2% vs 38.9%) and surgery was less common (8.9% vs 20.8%). Proactive TDM also decreased the odds of any unfavorable outcome by an odds ratio of 0.358. The authors concluded that "Proactive TDM is associated with fewer surgeries and higher rates of mucosal healing than conventional non-TDM-based management" (Fernandes et al., 2019).

Negoescu et al. (2019) performed a cost-effectiveness analysis of proactive verses reactive TDM in a simulated population of individuals with CD on IFX. The proactive strategy measured IFX concentration and antibody status every 6 months, at the time of a flare, then dosed IFX appropriately. The reactive strategy measured IFX concentration and antibodies at the time of a flare. The authors found that the proactive strategy led to fewer flares, finding an "incremental cost-effectiveness ratio of $146,494 per quality-adjusted life year." More patients stayed on IFX in the proactive strategy (63.4% vs 58.8% at year 5). The authors concluded that "assuming 40% of the average wholesale acquisition cost of biologic therapies, proactive TDM for IFX is marginally cost-effective compared with a reactive TDM strategy. As the cost of infliximab decreases, a proactive monitoring strategy is more cost-effective (Negoescu et al., 2019)."

Papamichael, et al. (2019) studied the therapeutic drug monitoring of adalimumab in populations with IBD. This multicenter retrospective cohort study included data from 382 patients with IBD (including 311 patients with CD). Participants received either standard or care, or at least one proactive TDM. "Multiple Cox regression analyses showed that at least one proactive TDM was independently associated with a reduced risk for treatment failure" (Papamichael, Juncadella, et al., 2019). This study shows that proactive TDM of adalimumab may help to decrease rates of treatment failure for IBD patients.

In February 2016, Guido et al. (2020) developed quality improvement (QI) methods to improve post-induction TDM in pediatric IBD patients initiating anti-TNF therapy at the Nationwide Children's Hospital in Columbus, OH. They implemented interventions to improve TDM using the Institute for Healthcare Improvement Plan-Do-Study-Act cycle approach. Their QI approaches improved post-induction anti-TNF TDM from a baseline off 43% in 2015 to greater than 80% by the end of 2017. Specifically, infliximab post-induction TDM and adalimumab post-induction TDM improved from a baseline of 59% to 89% and 14% to 79%, respectively. Most importantly, they note that "subtherapeutic post-induction infliximab levels were common, indicating a strong need for anti-TNF TDM and an opportunity for dose optimization."

Syversen et al. (2021) studied the therapeutic drug monitoring of infliximab in populations with immune-mediated inflammatory disease. Proactive therapeutic drug monitoring (TDM) as an alternative to standard therapies was proposed to treat patients safely and effectively during biologic drug therapies, specifically, in this study, patient populations who were prescribed Infliximab. A randomized, parallel-group and open-label clinical trial was established with a total of 458 adults with the diagnosis of rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, ulcerative colitis, Crohn disease, or psoriasis. All patients participating in Infliximab maintenance therapy were from a selection of Norwegian hospitals. Routine monitoring of serum drug levels and antidrug antibodies was performed on a randomized 1:1 basis (i.e. some patients received standard therapy, while others received scheduled monitoring of serum drug levels and anti-TNF antibodies.) The primary outcome of sustained disease control without disease worsening was evident in 167 patients, which comprised 73.6% of the therapeutic drug monitoring cohort. A total of 127 patients in the standard therapy group (55.9%) showed sustained disease control outcomes. This comprised an "estimated adjusted difference" of 17.6% between the two groups. In conclusion, the authors stated that they found "proactive TDM was more effective than treatment without TDM in sustaining disease control without disease worsening. Further research is needed to compare proactive TDM with reactive TDM, to assess the effects on long-term disease complications, and to evaluate the cost-effectiveness of this approach."

Cox et al. (2021) conducted a retrospective review of rheumatology patients who had antidrug antibody levels tested between October 2015 and April 2019 in order to assess the reasons for and outcomes in patients on adalimumab or infliximab. From the 237 patients included on the analysis, most patients were tested due to "clinical evidence of a flare in disease" and "patient reported worsening of symptoms." 38% changed biologics and 2% had dosing schedules changed, which is consistent with the 30-40% failure rate of response to first-line biologics. It was also found that "those with strongly positive antibodies were more likely to switch biologics than those with normal antibodies (84% vs 28%, p =0.01)," and that "patients with clinically active disease but normal antibodies and drug levels were more likely to switch biologics than patients with no evidence of active disease but positive antibodies (p=0.03)." This demonstrates the benefit of antidrug antibody level monitoring on informing treatment among specific patient populations (Cox et al., 2021).

Pan et al. (2022) utilized drug concentrations of infliximab, adalimumab, and ustekinumab in patients with postoperative Crohn's disease to investigate the impact on clinical outcomes. From 130 patients, the researchers found that in patients treated with infliximab with ≥3µg/mL and in patients treated with adalimumab ≥7.5µg/mL, "higher rates of deep remission existed," and similar differences were found for both clinical and objective remission. However, for ustekinumab, "clinical and objective remission were similar between patients regardless of drug concentration." These conclusions demonstrated that "established anti-tumor necrosis factor concentrations" could inform the rationale behind clinical

improvement for certain patients that suffer from diseases that lack prior data to support the positive use of bDMARDs (Pan et al., 2022).

V. Guidelines and Recommendations

National Institute for Health and Clinical Excellence (NICE)

The 2016 Guidelines for therapeutic monitoring of TNF-alpha inhibitors in Crohn's disease stated that "enzyme-linked immunosorbent assay (ELISA) kits show promise for therapeutic monitoring of tumor necrosis factor (TNF)-alpha inhibitors in people with Crohn's disease but there is insufficient evidence to recommend their routine adoption" (NICE, 2016).

NICE also states that use of ELISA tests should be used as part of research and/or data collection and that more research is needed to determine the clinical effectiveness of ELISA tests for therapeutic monitoring of TNF-alpha inhibitors for rheumatoid arthritis. "Enzyme-linked immunosorbent assay (ELISA) tests for therapeutic monitoring of tumour necrosis factor (TNF)-alpha inhibitors (drug serum levels and antidrug antibodies) show promise but there is currently insufficient evidence to recommend their routine adoption in rheumatoid arthritis. The ELISA tests covered by this guidance are Promonitor, IDKmonitor, LISA-TRACKER, RIDASCREEN, MabTrack, and tests used by Sanquin Diagnostic Services" (NICE, 2019).

American Gastroenterological Association (AGA)

The AGA published guidelines on Therapeutic Drug Monitoring in Inflammatory Bowel Disease recommending:

"In adults with active IBD treated with anti-TNF agents, the AGA suggests reactive therapeutic drug monitoring to guide treatment changes. Conditional recommendation, very low quality of evidence" (Feuerstein, Nguyen, Kupfer, Falck-Ytter, & Singh, 2017).

In adult patients with quiescent IBD treated with anti-TNF agents, the AGA makes no recommendation regarding the use of routine proactive therapeutic drug monitoring (Feuerstein et al., 2017).

A technical report released by the AGA in the same year noted that for patients with quiescent IBD being treated with anti-TNF agents, the benefit of routine proactive TDM was "uncertain" compared to no monitoring. However, they observe a potential benefit for reactive TDM (Vande Casteele et al., 2017).

American College of Rheumatology and National Psoriasis Foundation Guideline for the Treatment of Psoriatic Arthritis

These guidelines do not mention monitoring of TNF inhibitors for antidrug antibodies or TNF inhibitor levels (Singh et al., 2019).

American College of Gastroenterology (ACG)

The ACG released an update regarding management of Crohn's Disease (CD), stating that "if active CD is documented, then assessment of biologic drug levels and antidrug antibodies (therapeutic drug monitoring) should be considered" (Lichtenstein et al., 2018).

The ACG published guidelines on management of ulcerative colitis. In it, they observe that "the patient with nonresponse or loss of response to therapy should be assessed with therapeutic drug monitoring to identify the reason for lack of response and whether to optimize the existing therapy or to select an alternate therapy." However, they remark that there is "insufficient evidence" to support a benefit for proactive TDM in "all unselected patients with UC in remission" (Rubin et al., 2019).

Consensus Statement on Therapeutic Drug Monitoring of Biologic Agents for Patients With IBD

A consensus statement has been published on appropriate therapeutic drug monitoring for IBD patients. This statement was published in the journal of Clinical Gastroenterology and Hepatology, which is published by Elsevier on behalf of the AGA. A total of 28 statements were provided to a 13-member panel, and 24 of these statements reached a consensus. All statements were rated on a scale of 1-10, and statements were accepted if 80% or more of the participants agreed with a score ≥7. All 28 statements are shown below. Overall, "For anti-tumor necrosis factor (anti-TNF) therapies, proactive TDM was found to be appropriate after induction and at least once during maintenance therapy, but this was not the case for the other biologics. Reactive TDM was appropriate for all agents both for primary non-response and secondary loss of response. The panellists also agreed on several statements regarding TDM and appropriate drug and anti-drug antibody (ADA) concentration thresholds for biologics in specific clinical scenarios" (Papamichael, Cheifetz, et al., 2019).

"**Table 4:** Scenarios of Applying Therapeutic Drug Monitoring of Biological Therapy in Patients With Inflammatory Bowel Disease

Anti-TNFs

1. It is appropriate to order drug/antibody concentration testing in responders at the end of induction for all anti-TNFs. 92 (12/13)
2. It is appropriate to order drug/antibody concentration testing at least once during maintenance for patients on all anti-TNFs. 100 (13/13)
3. It is appropriate to order drug/antibody concentration testing of anti-TNFs at the end of induction in primary non-responders. 100 (13/13)
4. It is appropriate to order drug/antibody concentration testing for all anti-TNFs in patients with confirmed secondary loss of response. 100 (13/13)

Vedolizumab

5. It is appropriate to order drug/antibody concentration testing for vedolizumab in responders at the end of induction. 15 (2/13)a
6. It is appropriate to order drug/antibody concentration testing at least once during maintenance for patients on vedolizumab. 46 (6/13)a

7. It is appropriate to order drug/antibody concentration testing for vedolizumab in non-responders at the end of induction. 92 (12/13)
8. It is appropriate to order drug/antibody concentration testing for vedolizumab in patients with confirmed secondary loss of response. 83 (10/12)

Ustekinumab

9. It is appropriate to order drug/antibody concentration testing for ustekinumab in responders at the end of induction. 39 (5/13)a
10. It is appropriate to order drug/antibody concentration testing at least once during maintenance for patients on ustekinumab. 31 (4/13)a
11. It is appropriate to order drug/antibody concentration testing for ustekinumab in non-responders at the end of induction (at 8 weeks). 92 (12/13)
12. It is appropriate to order drug/antibody concentration testing for ustekinumab in patients with confirmed secondary loss of response. 83 (10/12) (Papamichael, Cheifetz, et al., 2019)"

Table 5: Biological Drug Concentrations and Anti-Drug Antibodies When Applying Therapeutic Drug Monitoring in Inflammatory Bowel Disease

General

13. There is no difference in indication for ordering drug/antibody concentrations or interpretation of results for biosimilars or the originator drug. 100 (13/13)
14. The threshold drug concentration may vary depending on disease phenotype and desired therapeutic outcome. 100 (13/13)
15. In the presence of adequate trough drug concentrations, anti-drug antibodies are unlikely to be clinically relevant. 100 (12/12)
16. Other than for anti-infliximab antibodies, there are not enough data to recommend a threshold for high anti-drug antibody titers for the biologic drugs. 100 (12/12)

Infliximab

17. The current evidence suggests that the variability of infliximab concentrations between the different assays is unlikely to be clinically significant. 100 (13/13)a
18. There is insufficient evidence that inter-assay drug concentration results are comparable for biologic drugs other than for infliximab. 100 (13/13)
19. The minimal trough concentration for infliximab post-induction at week 14 should be greater than 3 µg/mL, and concentrations greater than 7 µg/mL are associated with an increased likelihood of mucosal healing. 100 (13/13)
20. During maintenance the minimal trough concentration for infliximab for patients in remission should be greater than 3 µg/mL. For patients with active disease, infliximab should generally not be abandoned unless drug concentrations are greater than 10 µg/mL. 92 (12/13)
21. In the absence of detectable infliximab, high titer anti-infliximab antibodies require a change of therapy. Low level antibodies can sometimes be overcome. For the ANSER assay, a high titer anti-

infliximab antibody at trough is defined as 10 U/mL, for RIDAscreen the cutoff is 200 ng/mL, and for InformTx/Lisa Tracker the cutoff is 200 ng/mL. For other assays, there are insufficient data to define an adequate cutoff for a high titer anti-infliximab antibody. 100 (13/13)

Adalimumab

22. The minimum drug concentration at week 4 for adalimumab should at least be 5 µg/mL. Drug concentrations greater than 7 µg/ml are associated with an increased likelihood of mucosal healing. 83 (10/12)a
23. During maintenance the minimum trough concentration for adalimumab for patients in remission should be greater than 5 µg/mL. For patients with active disease, adalimumab should generally not be abandoned unless drug concentrations are greater than 10 µg/mL. 100 (12/12)

Certolizumab pegol

24. The minimum concentrations for certolizumab pegol at week 6 should be greater than 32 µg/mL. 100 (12/12)
25. During maintenance the minimum trough concentration for certolizumab pegol for patients in remission should be 15 µg/mL. 92 (11/12)

Golimumab

26. The minimum drug concentration at week 6 for golimumab should at least be 2.5 µg/mL. 92 (11/12)
27. During maintenance the minimum trough concentration for golimumab for patients in remission should be greater than 1 µg/mL. 92 (11/12)

Vedolizumab/Ustekinumab

28. Although there are emerging data that may show an association between drug concentrations and outcomes, they are not sufficient to guide specific induction and maintenance drug concentrations for vedolizumab and ustekinumab other than confirming that there is detectable drug. 100 (12/12) (Papamichael, Cheifetz, et al., 2019)"

International Association for Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT)

The IATDMCT have published guidelines to validate the use of dried blood spots (DBS) for the quantitative determination of small molecule drugs using chromatographic methods. This guideline is not focused on serum antibody testing methods, and do not mention monitoring of TNF inhibitors for antidrug antibodies or TNF inhibitor levels. (Capiau et al., 2019).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
80145	Adalimumab
82397	Chemiluminescent assay
80230	Infliximab
80280	Vedolizumab

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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In Vitro Chemoresistance and Chemosensitivity Assays

Policy #: AHS – G2100	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 1/19/22, 2/13/23 (See Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

In vitro chemotherapy sensitivity and resistance assays refer to any in vitro laboratory analysis that is performed specifically to evaluate whether tumor growth is inhibited by a known chemotherapy drug or, more commonly, a panel of drugs (Hatok et al., 2009; Schrag et al., 2004).

II. Related Policies

Policy Number	Policy Title
	Not applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

1. In vitro chemosensitivity assays, including, but not limited to, the histoculture drug response assay or a fluorescent cytoprint assay, **DO NOT MEET COVERAGE CRITERIA.**

2. In vitro chemoresistance assays, including, but not limited to, extreme drug resistance (EDR) assays, **DO NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Chemotherapy treatment recommendation has long been based on carefully designed clinical studies in large patient populations and provide an individual patient with a probability for response based on clinically observed response rates. This approach has led to major progress in clinical oncology and has helped to identify successful therapeutic regimens for patients with many cancers. However, the response rates are relatively low, and there are still many cancers for which there is only marginal treatment. Tumor cells isolated from these patients often are resistant to a wide range of anticancer drugs. In addition, it is becoming clear that each individual patient's tumor is genotypically and phenotypically different (Hatok et al., 2009).

Chemotherapy sensitivity and resistance assays were developed to determine if a patient with cancer might be resistant or sensitive to a specific chemotherapy treatment prior to use. A chemosensitivity assay detects the effects (cytotoxic, apoptotic, and so on) of a given chemotherapeutic agent outside an organism. The assays vary, but typically they follow the same steps: cells from the patient are isolated, incubated with the chemotherapeutic agent, and assessed for cell survival and cell response (Hatok et al., 2009; Tatar et al., 2016). This assay allows clinicians to evaluate the effects of the chemotherapeutic agent without unnecessary exposure to cells. However, there are difficulties with these assays; for example, the potency of a chemotherapeutic agent may only be seen after time has elapsed. Many assays have been created to assess the potency of chemotherapeutic agents, including proprietary tests such as ChemoFX and ChemoINTEL, as well as non-proprietary assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolyum bromide (MTT), adenosine triphosphate-tumor chemosensitivity (ATP-TCA), and differential staining cytotoxicity (DISC) (Tatar et al., 2016).

Chemosensitive assays typically rely on the use of cell cultures within the presence of the anticancer agent(s). For example, the MTT procedure involves culturing tumor cells with anticancer agents, then adding MTT, which is reduced to a blue dye in the cell. The intensity of the uptake allows the user to estimate the drug resistance of the tumor cells. DISC cultures tumor cells in three different concentrations of the drug, incubates them for 6 days, then uses differential dye staining to identify viable cells (Hatok et al., 2009). Several proprietary assays exist, such as ChemoFX (from Precision Therapeutics now merged with Helomics), which exposes tumor cells to increasing doses of chemotherapeutic drugs, and the number of live cells remaining post-treatment is counted. These counts are combined into a dose-response curve, which is used to categorize a tumor's response as "responsive," "intermediate response," or "non-responsive" (Brower, Fensterer, & Bush, 2008). Another proprietary test is the Microculture-Kinetic (MiCK) assay (from DiaTech Oncology, now Pierian) (Grendys et al., 2014). This test relies on drug-induced apoptosis with the quantification of tumor cells' response to chemotherapeutic agents. This test is now branded as ChemoINTEL (Pierian, 2019). A third proprietary test comes from RGCC, titled "Onconomics". This test evaluates both molecular markers and viability assessments to determine efficacy of certain drugs. However, this test does follow the same pattern as the previously discussed tests; developing cell cultures and examining effects of chemotherapeutic agents on their population (RGCC, 2020).

Clinical Utility and Validity

Tatar et al. (2016) conducted a study to assess three in vitro chemosensitivity assays in ovarian carcinoma. 26 patients with ovarian carcinoma contributed tumoral tissue, and three assays (the MTT assay, the ATP-TCA assay, and the DISC assay) were used to evaluate the chemosensitivity of paclitaxel, carboplatin, docetaxel, topotecan, gemcitabine, and doxorubicin. The authors stated that all three assays correlated reasonably well with each other and are "particularly useful for serous and advanced cancers". However, they caution that "large prospective studies comparing standard versus assay-directed therapy with an endpoint of overall survival are required before routine clinical utilization of these assays" (Tatar et al., 2016).

Kwon et al. (2016) evaluated the usefulness of the in vitro adenosine triphosphate-based chemotherapy response assay (ATP-CRA) for prediction of clinical response to fluorouracil-based adjuvant chemotherapy in stage II colorectal cancer. Tumor specimens of 86 patients with stage II colorectal adenocarcinoma were tested for chemosensitivity to fluorouracil, and chemosensitivity was determined by cell death rate (CDR) of the drug-exposed cells. 11 of the 86 patients had a recurrence, and the group with CDR ≥20% was associated with better disease-free survival than the group under 20%. The authors concluded that "in stage II colorectal cancer, the in vitro ATP-CRA may be useful in identifying patients likely to benefit from fluorouracil-based adjuvant chemotherapy" (Kwon et al., 2016).

Krivak et al. (2014) conducted an observational study to evaluate if the ChemoFx assay can identify patients who are platinum-resistant prior to treatment. The study included 276 women with International Federation of Gynecology and Obstetrics stage III-IV ovarian, fallopian, and peritoneal cancer, and the responsiveness of their tumors was evaluated. All patients were treated with a platinum/taxane regimen following cytoreductive surgery. The authors found that the patients whose tumors were resistant to carboplatin were at increased risk of disease progression compared to those who were nonresistant. The authors stated that "assay resistance to carboplatin is strongly associated with shortened PFS among advanced-stage epithelial ovarian cancer patients treated with carboplatin + paclitaxel therapy, supporting use of this assay [ChemoFx] to identify patients likely to experience early recurrence on standard platinum-based therapy" (Krivak et al., 2014).

Rutherford et al. (2013) conducted a prospective study evaluating the use of ChemoFx assay in recurrent ovarian cancer patients. The study included 252 women with persistent or recurrent ovarian cancer and fresh tissue samples were collected for chemoresponse testing. Patients were treated with one of 15 protocol-designated treatments empirically selected by the oncologist, blinded to the assay results. Patients were prospectively monitored for progression-free survival (PFS) and overall survival (OS). Patients treated with an assay-sensitive regimen demonstrated significantly improved PFS and OS while there was no difference in clinical outcomes between intermediate and resistant groups. The researchers concluded that the "study demonstrated improved PFS and OS for patients with either platinum-sensitive or platinum-resistant recurrent ovarian cancer treated with assay-sensitive agents" (Rutherford et al., 2013).

Hoffman (2018) conducted a study investigating the clinical correlation of histoculture drug response assay (HDRA) in 29 advanced gastric and colon cancer patients. The authors revealed that all 29 were being treated with drugs considered "ineffective" by the HDRA. However, nine patients were also being treated with drugs identified as "effective" by the HDRA, and these patients showed response or arrest of disease progression. The authors investigated another subset of 32 patients treated with mitomycin C and 5-fluorouracil (5-FU) and whom had advanced gastric cancer. Ten patients were identified as

"sensitive" to these drugs, and their survival rates were higher than the other 22 whose tumors were "insensitive". A separate 128-patient subset had their tumors evaluated by the HDRA, and the overall and disease-free survival rate was higher for the sensitive group compared to the resistant group. Overall, both "sensitive" groups experienced higher survival rates (Hoffman, 2018).

Strickland et al. evaluated the correlation of the MiCK assay with patient outcomes in initial treatment of adult acute myelocytic leukemia (AML). 109 patients with untreated AML contributed samples for the MiCK assay. The amount of apoptosis was measured over 48 hours and standardized to "kinetic units" of apoptosis (KU). The authors observed that complete remission (CR) was "significantly" higher in patients with high idarubicin-induced apoptosis (>3 KU) compared to patients with <3 KU. A multivariate analysis indicated the only significant variable to be idarubicin-induced apoptosis. The authors concluded, "Chemotherapy-induced apoptosis measured by the MiCK assay demonstrated significant correlation with outcomes and appears predictive of complete remission and overall survival for patients receiving standard induction chemotherapy" (Strickland et al., 2013).

Howard et al. developed and assessed a "chemopredictive" assay (ChemoID), which was intended to identify the most effective chemotherapy out of a panel of selected treatments. ChemoID evaluates the efficacy of chemotherapies using a patient's live tumor cells, as well as the cancer stem cells (CSC) that are purported to cause recurrence in patients. The study included 42 glioblastoma who were treated with standard of card temozolomide (TMZ). Clinical outcomes, such as "tumor response, time to recurrence, progression-free survival (PFS), and overall survival (OS). Odds ratio (OR) associations of 12-month recurrence, PFS, and OS outcomes," were estimated. The authors found that for every 5% increase in CSC kill by TMZ, 12-month patient response (defined as "nonrecurrence of cancer") increased by 2.2-fold. The authors also identified a less significant association with the bulk tumor cells; a 5% increase in bulk tumor cell kill corresponded with a 2.75-fold increase in nonresponse ($p = .07$). At >40% cell kill for CSC and >55% cell kill for bulk tumor cells, the area under curve was 0.989. Median recurrence time was 20 months for patients with a positive (defined as >40%) CSC test, compared to 3 months for patients with a negative test. Similarly, median recurrence time was 13 months for patients with a positive bulk tumor cell test (>55%), compared to 4 months for a negative test. Finally, the ChemoID CSC results were found to "potentially" identify more optimal treatments in 34 patients, while the bulk tumor results may have resulted in more optimal treatments in 27 patients. Overall, the authors concluded that "the ChemoID CSC drug response assay has the potential to increase the accuracy of bulk tumor assays to help guide individualized chemotherapy choices" (Howard et al., 2017).

Chen et al. (2018) evaluated in vitro chemosensitivity and multiple drug resistance (MDR) using an ATP-based tumor chemosensitivity assay (ATP-TCA). The authors evaluated 120 lung cancer patients' chemosensitivity to eight single drug chemotherapies and 291 lung cancer patients' chemosensitivity to seven chemotherapy regimens. Additionally, 284 lung adenocarcinoma patients and 90 lung squamous cell carcinoma patients were evaluated for chemosensitivity to both single-drug and chemotherapy regimens. Authors found that "PTX (51.7%), TXT (43.3%), GEM (12.5%), PTX+DDP (62.5%), TXT+L-OHP (54.3%) and VP-16+DDP (16.2%) had the highest in vitro chemosensitivity rates." Additionally, approximately 37.1% of patients developed resistance to eight single-drug chemotherapies; 25.8% showed resistance to all seven chemotherapy regimens. In conclusion, testing for drug sensitivity before chemotherapy could assist in preventing the "occurrence of primary drug resistance and inappropriate drug treatment" (Chen et al., 2018).

V. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

The 2011 clinical practice guideline update states that: "The use of chemotherapy sensitivity and resistance assays to select chemotherapeutic agents for individual patients is not recommended outside of the clinical trial setting. Oncologists should make chemotherapy treatment recommendations on the basis of published reports of clinical trials and a patient's health status and treatment preferences. Because the in-vitro analytic strategy has potential importance, participation in clinical trials evaluating these technologies remains a priority" (Burstein et al., 2011).

National Comprehensive Cancer Network (NCCN)

The NCCN Practice Guidelines in Oncology for Ovarian Cancer (NCCN, 2020b) state that: "chemosensitivity/resistance and/or other biomarker assays are being used at some NCCN Member Institutions for decisions related to future chemotherapy in situations where there are multiple equivalent chemotherapy options available. The current level of evidence is not sufficient to supplant standard of care chemotherapy". This is a category 3 recommendation (based on any level of evidence but reflects major disagreement).

Chemosensitivity/resistance testing is not mentioned in the guidelines for gastric, colon, or prostate cancers (NCCN, 2020a).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81535	Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain and morphology, predictive algorithm reported as a drug response score; first single drug or drug combination
81536	Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain and morphology, predictive algorithm reported as a drug response score; each additional single drug or drug combination (List separately in addition to code for primary procedure)
88104	Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation
88305	Level IV - surgical pathology, gross and microscopic examination

88313	Special stain including interpretation and report; Group II, all other (e.g., iron, trichrome), except stain for microorganisms, stains for enzyme constituents, or immunocytochemistry and immunohistochemistry
88358	Morphometric analysis; tumor (e.g., DNA ploidy)
89050	Cell count, miscellaneous body fluids (e.g., cerebrospinal fluid, joint fluid), except blood;
0083U	Oncology, response to chemotherapy drugs using motility contrast tomography, fresh or frozen tissue, reported as likelihood of sensitivity or resistance to drugs or drug combinations Proprietary test: Onco4D™ Lab/manufacturer: Animated Dynamics, Inc.
0248U	Oncology (brain), spheroid cell culture in a 3D microenvironment, 12 drug panel, tumor-response prediction for each drug Proprietary test: 3D Predict Glioma Lab/Manufacturer: KIYATEC®, Inc
0249U	Oncology (breast), semiquantitative analysis of 32 phosphoproteins and protein analytes, includes laser capture microdissection, with algorithmic analysis and interpretative report
0324U	Oncology (ovarian), spheroid cell culture, 4-drug panel (carboplatin, doxorubicin, gemcitabine, paclitaxel), tumor chemotherapy response prediction for each drug
0325U	Oncology (ovarian), spheroid cell culture, poly (ADP-ribose) polymerase (PARP) inhibitors (niraparib, olaparib, rucaparib, velparib), tumor response prediction for each drug
0564T	Oncology, chemotherapeutic drug cytotoxicity assay of cancer stem cells (CSCs), from cultured CSCs and primary tumor cells, categorical drug response reported based on percent of cytotoxicity observed, a minimum of 14 drugs or drug combinations (Reported for ChemoID®)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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IX. Revision History

Revision Date	Summary of Changes
January 19, 2022	Added CPT code **0564T** (Oncology, chemotherapeutic drug cytotoxicity assay of cancer stem cells [CSCs], from cultured CSCs and primary tumor cells, categorical drug response reported based on percent of cytotoxicity observed, a minimum of 14 drugs or drug combinations [Reported for ChemoID®]), which is a not-covered code with SelectHealth
February 13, 2023	Added the following CPT codes, which are all not-covered codes with SelectHealth: **0249U** Oncology (breast), semiquantitative analysis of 32 phosphoproteins and protein analytes, includes laser capture microdissection, with algorithmic analysis and interpretative report **0324U** Oncology (ovarian), spheroid cell culture, 4-drug panel (carboplatin, doxorubicin, gemcitabine, paclitaxel), tumor chemotherapy response prediction for each drug **0325U** Oncology (ovarian), spheroid cell culture, poly (ADP-ribose) polymerase (PARP) inhibitors (niraparib, olaparib, rucaparib, velparib), tumor response prediction for each drug

Intracellular Micronutrient Analysis

Policy #: AHS – G2099	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Micronutrients are dietary components, often referred to as vitamins and minerals, which although only required by the body in small amounts, are vital to development, disease prevention, and wellbeing. Micronutrients are not produced in the body and must be derived from the diet (CDC, 2015; Life, 2012). Micronutrients include essential trace elements such as boron, iron, zinc, selenium, manganese, iodine, copper, molybdenum, cobalt, and chromium (Frieden, 1985; WHO, 1973), and essential vitamins such as vitamins A, B, C, D, and K (organic) (Gidden & Shenkin, 2000).

II. Related Policies

Policy Number	Policy Title
AHS-G2056	Diagnosis 0f Idiopathic Environmental Intolerance

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

1. Intracellular micronutrient panel testing, including but not limited to SpectraCell, Cell Science Systems cell micronutrient assay and ExaTest, **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Micronutrients, such as zinc, selenium, and copper, are involved in metabolic processes, either as catalysts or facilitators for various enzymatic functions. Micronutrient deficiency can result from general malnutrition, a current illness, or side effects of medications or procedures. Nutritional loss may exacerbate severe illness and side effects of medications as the inflammatory response draws micronutrients to the damaged organs, causing an increase in oxidative stress, and normal defense mechanisms to fail (Preiser et al., 2015). For example, oxidative damage in copper deficiency results in muscle weakness and edema, and impaired oxidative status in iodine deficiency leads to a decrease in thyroid hormone synthesis and mental retardation (Pazirandeh, 2020; Pearce, Lazarus, Moreno-Reyes, & Zimmermann, 2016).

The measurement of serum vitamin and mineral levels is widely available from numerous commercial testing companies. Normal serum nutrient concentration varies based on its function in the body. Serum concentrations of nutrients involved in regulatory mechanisms, such as calcium and zinc, are maintained within narrow ranges regardless of body stores and any changes only occur with severe nutrient deficiency. Other nutrients, such as carotenoids, vary in the body depending on recent intake or half-life length. Environmental factors, such as infections or stress, can also influence serum nutrient concentrations. Vitamin C, Vitamin B, selenium, and magnesium play a role in reducing the levels of cortisol and adrenalin in the body (McCabe, Lisy, Lockwood, & Colbeck, 2017). Nutrient concentrations may also vary based on the tissue. Nutrient concentrations in cell membranes or bone fluctuate less, but these measurements are more difficult to obtain (Elmadfa & Meyer, 2014). Serum nutrient testing is promoted to the public as a nutrient deficiency screening and supplement personalization, but these tests are usually unwarranted. There is not enough information available regarding the optimal blood levels of vitamins. Moreover, there is a lack of evidence that vitamin supplements prevent disease in healthy adults with low blood levels of vitamins, apart from those with specific diets or conditions. Vitamin deficiencies typically occur in special populations such as the elderly or those with gastric bypass surgery, and not the general public (Fairfield, 2017).

Another possible method of measuring nutrient deficiency is to assess the intracellular concentration (as opposed to the typical serum measurement). Intracellular micronutrient lymphocyte analysis was developed based on the premise that a peripheral blood lymphocyte reflects the genetic and biochemical state of the person at the time it was formed (Shive et al., 1986). A study was performed to validate the measurement of lymphocytes as an indicator of an individual's nutrient state. Lymphocytes were hypothesized to provide a superior history of nutritional status rather than a "snapshot" from typical serum testing as proclaimed by the authors. Lymphocytes were grown in various chemically defined serum-free media, and their growth responses were measured. This lymphocyte growth response was used as an indicator of nutritional status. The authors concluded that lymphocytes provide an accurate method of determining nutrient needs, requirements, or deficiencies (Bucci, 1993, 1994).

Lymphocyte measurement is the basis of SpectraCell's micronutrient testing procedure. Lymphocytes are isolated from the blood sample and placed in a culture medium containing the optimal levels of nutrients for sustained growth. A given micronutrient is removed, and then growth is measured and

compared against the 100% level of growth. For example, Vitamin B6 may be removed from the medium. The growth rate of the cell is theoretically only dependent on vitamin B6 as all other micronutrients are at optimal levels; therefore, any deficiency in cell growth would be caused by issues with intracellular Vitamin B6. This is done for all 31 micronutrients in the panel and results are reported. The micronutrients included in SpectraCell's panels are as follows: Vitamins A, B1, B2, B3, B6, B12, C, D, E, and K, as well as biotin, folate, pantothenate, calcium, magnesium, manganese, zinc, copper, asparagine, glutamine, serine, oleic acid, alpha-lipoic acid, coenzyme Q10, cysteine, glutathione, selenium, chromium, choline, inositol, and carnitine. SpectraCell also provides an assessment of "Total Antioxidant Function," an "Immune Response Score," and measures of fructose sensitivity and glucose-insulin metabolism (SpectraCell, 2021b).

Another test analyzing intracellular concentration is ExaTest by IntraCellular Diagnostics. From their laboratory website, this test uses "rapidly metabolizing sublingual epithelial cells under Analytical Scanning Electron Microscopy, (ASEM) an Energy Dispersive X-Ray Analysis, (EXA) to reflect fast tissue changes of vital mineral electrolytes." This test is primarily for aid with the management of heart disease and provides tissue evaluations of magnesium, sodium, calcium, phosphorus, potassium, and chloride. ExaTest proclaims its ability to follow a patient's metabolic status and assess electrolyte imbalance easily. First, the buccal, epithelial cells are swabbed from the patient. Then the sample is analyzed by the proprietary energy dispersive x-ray analysis and bombarded with X-Rays. Energy is released by wavelengths (unique to each element), and the element composition is analyzed and reported. ExaTest states that the serum or urine of some minerals do not correlate with intracellular levels and that these deficiencies are common in patients with various health issues, particularly heart disease. Buccal cells are used as they are easily accessible and have an easily analyzed structure for electrolytes (Exatest, 2014).

Vibrant America has also developed a test that gives both extracellular and intracellular information on approximately 40 vitamins, minerals, amino acids, fatty acids and antioxidants in the body (Vibrant, 2017). Vibrant America states that the benefits of intracellular testing include the identification of potential functional deficiencies in the cellular nutrient absorption process (which may increase the risk of certain diseases), and the identification of an individual's nutritional status in the previous four to six months (Vibrant, 2017).

While limited research has been completed regarding intracellular micronutrient lymphocyte analysis, Yamada, Yamada, Waki, and Umegaki (2004) did complete a study with 41 type 2 diabetes patients and 50 healthy controls. No participants were taking vitamin supplements at the time of the study. Blood samples were taken from all participants during a fasting state; the researchers determined that the lymphocyte vitamin C level was significantly lower in the type 2 diabetes patients than in controls (Yamada et al., 2004). This study may support the above theory that lymphocytes can be used as an indicator of an individual's nutrient state.

Houston (2010) published a small study stating that treating the intracellular micronutrient deficiencies in combination with optimal diet, exercise and other weight management resulted in reaching blood pressure goals for 62% of a hypertensive population (Houston, 2010). Another small study of 10 patients found that both genders showed overall improvement in their vitamin and mineral cellular storage balance after being tested with SpectraCell's assessment (Frye, 2010). However, the authors of each of the aforementioned studies (Houston, Bucci, Frye, and Shive) are associated with SpectraCell Laboratories. SpectraCell has listed several studies on their website discussing serum versus intracellular

deficiencies; from discussing the effect of the inflammatory response on serum micronutrient levels to Vitamin B12's difficult serum profile to micronutrient deficiencies in special populations (SpectraCell, 2021a). However, none of these studies reported use SpectraCell's actual method as of 2018, nor did the studies cover the healthy population for which the test is marketed. Most of these studies listed used other methods such as HPLC to measure micronutrient levels instead of the proprietary method provided by SpectraCell. Few other studies listed on SpectraCell's website used lymphocytes as the analyte as well.

Another possible method of analyzing nutrient deficiency is by measuring lymphocyte proliferation in response to micronutrient concentration. Cell Science Systems (CSS) released a cellular micronutrient assay (CMA) which measures the effect of micronutrients on lymphocyte proliferation when stimulated with a mitogen. According to their protocol, lymphocytes are primarily separated from the patient's whole blood and the patient's own serum is added back to the lymphocytes. The cells are stimulated with a mitogen and baseline lymphocyte proliferation rates (without the addition of micronutrients) are recorded. Next, micronutrients are added to the lymphocyte culture and proliferation rates are compared to the baseline rate. If the addition of micronutrients to the lymphocyte culture enhances lymphocyte proliferation, a nutrient insufficiency is reported. If the lymphocyte proliferation rate with the addition of micronutrients does not exceed the baseline rate, it likely indicates sufficient stores of that nutrient. The CMA measures vitamins, amino acids, minerals, and other nutrients such as carnitine, alpha-ketoglutarate, choline, glutathione, and inositol. By measuring intracellular levels of micronutrients, the test is intended to provide insight into the long-term nutritional status (6 months) versus the short term variability of serum nutrient levels, which is prone to daily fluctuations (Cell_Science_Systems, 2020).

In a randomized observational analysis, the Cell Science Systems (CSS) cellular micronutrient assay (CMA) was used to examine nutritional status in 845 American individuals aged 13 years and older. Results were expressed as the stimulation index (SI), which is the percentage of lymphocyte stimulation in response to the mitogen. All subjects were divided into two groups based on their diet. The first group had a healthy diet, consisting of whole fresh foods including fruits, vegetables, nuts, while the poor diet group reported high consumption of sweets, fried, frozen, and starchy foods. CMA analysis indicated that the "mean values for micronutrient deficiency were significantly higher in the poor diet group as compared to the healthy diet group with p-values of 0.0017 and 0.0395, respectively." According to the authors, "the adequate functioning of this defensive system is critically impacted by intracellular nutritional status, and its interaction with the host' cells. Lacking adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response" (Steele, Allright, & Deutsch, 2020).

V. Guidelines and Recommendations

No studies evaluating the accuracy or clinical utility of intracellular micronutrient testing compared to standard testing for vitamin or mineral levels were identified. In addition, no controlled studies that evaluated changes to patient management or health impact of intracellular micronutrient testing were identified. Limited data are available on correlations between serum and intracellular micronutrient levels. Intracellular micronutrient analysis was not included in reviews on micronutrient analysis (Elmadfa & Meyer, 2014; Raghavan, Ashour, & Bailey, 2016).

No recommendations or practice guidelines recommending intracellular micronutrient testing were identified in a literature search.

VI. State and Federal Regulations (as applicable)

Intracellular micronutrient testing is offered by companies SpectraCell, IntraCellular Diagnostics, and Cell Science Systems Corporation which have Clinical Laboratories Improvement Amendments (CLIA) accredited laboratories. SpectraCell's micronutrient panel test, the IntraCellular Diagnostics ExaTest, and the Cell Science Systems Cellular Micronutrient Assay (CMA) have not been through the FDA approval process. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82128	Amino acids; multiple, qualitative, each specimen
82136	Amino acids, 2 to 5 amino acids, quantitative, each specimen
82180	Ascorbic acid (vitamin c), blood
82310	Calcium; total
82379	Carnitine (total and free), quantitative each specimen
82495	Chromium
82525	Copper
82607	Cyanocobalamin (Vitamin B-12);
82652	Vitamin D; 1, 25 dihydroxy, includes fraction(s), if performed
82725	Fatty acids, nonesterified
82746	Folic acid; serum
82978	Glutathione
83735	Magnesium
83785	Manganese
84207	Pyridoxal phosphate (vitamin b-6)
84252	Riboflavin (vitamin b-2)
84255	Selenium
84425	Thiamine (vitamin b-1)
84446	Tocopherol alpha (Vitamin E)
84590	Vitamin A
84591	Vitamin, not otherwise specified

Code Number	Code Description
84597	Vitamin K
84630	Zinc
86353	Lymphocyte transformation, mitogen (phytomitogen) or antigen induced blastogenesis
88348	Electron microscopy, diagnostic

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes

Laboratory Procedures Reimbursement Policy

Policy #: AHS – R2162	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Scope

To be considered for reimbursement, all outpatient laboratory claims should be submitted in accordance with:

- AMA CPT and HCPCS coding and ICD-10 diagnosis coding guidelines
- Other laboratory and pathology coding guidelines
- All applicable regulatory guidelines

This policy outlines additional requirements beyond the guidelines listed above that are required for reimbursement. Note that these guidelines are reviewed and updated periodically.

II. Modifier Guidelines/Instructions

Technical, Professional, and Global services (TC, 26 modifiers)

- Before using the 26 or TC modifiers, verify that these modifiers are allowable with the procedure code.
- Do not append these modifiers to the procedure code when performing the global service.

Tests performed by a Reference laboratory

- When laboratory procedures are performed by a party other than the treating or reporting physician to other qualified health care professional, the procedure must be identified by adding modifier 90 to the claim line.
- Only independent clinical laboratories may append modifier 90 to indicate that the service was referred to an outside laboratory.

Repeat Testing

- While treating a patient, it may be necessary to repeat the same laboratory test on the same day to obtain subsequent (multiple) test results. Under these circumstances, the laboratory test performed can be identified by its usual procedure number and the addition of modifier 91.

- Modifier 91 may not be used when tests are rerun to confirm initial results; due to testing problems with specimens or equipment; or for any other reason when a normal, one-time, reportable result is all that is required.
- Modifier 91 may not be used when other code(s) describe a series of test results (e.g., glucose tolerance tests, evocative/suppression testing).

Clinical Laboratory Improvement Amendments (CLIA) Waived Testing

- Laboratory tests which are CLIA-waived must have the QW modifier appended to the procedure code.

III. Place of Service Guidelines

In accordance with S611b of OBRA of 1989, a referring lab can bill for tests performed by a reference lab only if it meets any one of the following exceptions:

- The referring laboratory is in or is part of a rural hospital
- The referring lab and the reference lab are 'subsidiary related.' That is:
  - **The referring lab is a wholly owned subsidiary of the reference lab**
  - **The referring lab wholly owns the reference lab**
  - **Both the referring lab and reference lab are wholly owned subsidiaries of the same entity.**

IV. Non-Reimbursable CPT/HCPCS Codes

Some procedure codes will not be reimbursed due to their expiration or replacement with more appropriate codes.

- AMA drug assay codes 80320 to 80377 are not accepted and will not be reimbursed. Refer to policy T2015, Opioids Testing in Pain Management and Substance Abuse, for guidelines for submitting G0480 to G0483.
- Proprietary Laboratory Analyses (PLA) codes will not be reimbursed unless a laboratory policy specifically covers the PLA code.
- Unlisted codes (81479, 81599, 84999) will not be accepted if a specific Tier 1, Tier 2, GSP, or MAAA code exists.

V. Edit Types

Outpatient lab claims are consistently evaluated for reimbursement against several standard edit types using administrative information (e.g., claim information, historical claims). The specific edits are described below.

Additional Tests on the Date of Service

The presence or absence of additional tests on a single date of service (DOS) may trigger a reimbursement denial for a claim line.

The exclusivity edit is based upon:

- A list of tests where Correct Coding Initiative (CCI) and/or AMA coding guidance identify that two procedure codes for the test are not permitted for the same patient at the same time because it is only appropriate to charge for one of those procedures.
- Clinical guidelines for testing preclude the simultaneous performing of two tests. For example, individual components of panel procedures codes will not be separately reimbursed when billed with the panel procedure code.
- Technically complex procedures which incorporate simple procedures will not be reimbursed for the same patient on the same DOS. For example, billing for multiple testing methodologies (e.g., direct, amplification, and quantitative testing) for the same microorganism codes is not reimbursed.

Thus, a denial based upon this edit is one that is based upon evaluation of universal, objective criteria related to how the test is being billed, not an assessment of a patient's condition to determine whether both codes were appropriate.

Incorrect Diagnosis Code

Select diagnosis and procedure code combinations are permitted or precluded depending on the nature of the policy.

The edit functions to identify those tests that are never appropriate unless the physician has first concluded that the patient presents with the indicated diagnosis. Although the edit is contingent upon the diagnosis of the individual patient, it is not conducting any clinical evaluation of whether the condition, in fact, exists. Rather, the inherent nature of the test (only being indicated for patients with the condition or contraindicated for the condition) and the question of whether the pre-requisite condition is present are the conditions for reimbursement.

Incorrect Patient Age

This edit addresses medical policies with coverage criteria, CPT/HCPCS codes, and diagnosis codes that not are reimbursable based on the patient's age on the DOS.

For example, testing on newborns must be associated with a member who is 28 days of age or younger.

Incorrect Place of Service

This edit is invoked when the Place of Service is identified as inappropriate with the laboratory test/service performed submitted on the claim.

Once per Lifetime Tests

This edit limits the frequency of applicable laboratory services/procedure codes to once in the patient's lifetime.

Certain laboratory services should only be performed once in a patient's lifetime as outlined in medical policy. If a once-per-lifetime test is submitted for reimbursement more than once, the subsequent submissions will not be reimbursed.

Unit Threshold Met (Daily and Historical)

These edits are invoked when the number of units billed for the procedure on a single DOS or over a period of time exceed an allowed reimbursement quantity without considering any aspect of an individual's specific condition. Maximum units of service are determined by one or more of the following:

- The CPT or HCPCS code description defines the number of units per patient per DOS for a unique billing event.
- Laboratory Coverage Guidelines outlined in medical policy establish the number of units for a laboratory service.
- The service is anatomically or clinically limited to the number of procedures that may be performed and therefore units billed.
- Scientific or statistical analyses demonstrate a reasonable limitation of the number of units that should be performed within a period of time.
- Third parties, such as Correct Coding Initiative or Centers for Medicare and Medicaid Services, limit reimbursement to a specified number of units.

If a procedure code that is assigned a maximum unit value is reported with a greater unit count, the claim line will be reimbursed only for the number of units up to but not exceeding the allowed maximum.

VI. References

1. Centers for Medicare and Medicaid Services, "Medically Unlikely Edits" https://www.cms.gov/Medicare/Coding/NationalCorrectCodInitEd/MUE.html
2. American Medical Association, Current Procedural Terminology (CPT ®), Professional Edition
3. https://www.cms.gov/Regulations-and-Guidance/Guidance/Manuals/downloads/clm104c16.pdf
4. CMS Pub. 100-04, chapter 16, section 40.1.1 external link (PDF, 497 KB)

VII. Revision History

Revision Date	Summary of Changes

Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease

Policy #: AHS – G2121	**Prior Policy Name & Number (as applicable):** G2121 – Serum Antibodies for the Diagnosis of Inflammatory Bowel Disease
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Inflammatory bowel disease (IBD) is a class of inflammatory bowel disorders comprised of two major disorders: ulcerative colitis and Crohn's disease each with distinct pathologic and clinical characteristics (Peppercorn & Cheifetz, 2021).

Ulcerative colitis (UC) is a chronic inflammatory condition characterized by relapsing and remitting episodes of inflammation limited to the mucosal layer of the colon (Silverberg et al., 2005) beginning at the rectum and may extend in a proximal and continuous fashion to involve other parts of the colon (Peppercorn & Kane, 2022b).

Crohn's disease (CD) is characterized by patchy transmural inflammation (skip lesions) of the gastrointestinal tract resulting in sinus tracts, and ultimately microperforations and fistulae (Silverberg et al., 2005). It may also lead to fibrosis, strictures and to obstructive clinical presentations that are not typically seen in ulcerative colitis (Gasche et al., 2000; Peppercorn & Kane, 2022a).

II. Related Policies

Policy Number	Policy Title
AHS-G2043	Celiac Disease Testing
AHS-G2060	Fecal Analysis in The Diagnosis of Intestinal Dysbiosis
AHS-G2061	Fecal Calprotectin Testing
AHS-G2155	General Inflammation Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

1) For the workup and monitoring of individuals with inflammatory bowel disease (IBD), the use of serologic markers, including, but not limited to, the following, **DOES NOT MEET COVERAGE CRITERIA**:
   a) Anti-neutrophil cytoplasmic antibody (ANCA).
   b) Anti-*Saccharomyces cerevisiae* antibody (ASCA).
   c) Perinuclear anti-neutrophilic cytoplasmic antibody (pANCA).
   d) Antibody to Escherichia coli outer membrane porin C (anti-OmpC).
   e) Antibody to Pseudomonas fluorescens-associated sequence I2 (anti-I2).
   f) Anti-CBir1 flagellin antibody (anti-cBir1).
   g) Antichitobioside antibodies (ACCA IgA).
   h) Antilaminaribioside antibodies (ALCA IgG).
   i) Antimannobioside antibodies (AMCA IgG).
   j) Pyruvate kinase M2 (PKM2).
2) For the diagnosis or monitoring of individuals with IBD, the use of diagnostic algorithm-based testing, including testing that combines serologic, genetic, and inflammation markers (such as Prometheus® testing), **DOES NOT MEET COVERAGE CRITERIA.**
3) Genetic testing for IBD **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

The diagnoses of Crohn's disease (CD) and ulcerative colitis (UC) depend on a combination of clinical, laboratory, radiographic, endoscopic, and histological criteria. Differential diagnosis can be challenging but is highly important toward treatment and prognosis. Serological markers could be of value in differentiating CD from UC, in cases of indeterminate colitis, and in predicting the disease course of IBD (Peppercorn & Cheifetz, 2021; Peppercorn & Kane, 2022a, 2022b).

Investigations based on animal models have led to the current theory that chronic intestinal inflammation is the result of an aberrant immunologic response to commensal bacteria within the gut lumen (Blumberg et al., 1999; Strober et al., 2002). Immune responses toward commensal enteric organisms have been investigated in CD and UC (Akasaka et al., 2015; D'Haens et al., 1998). Patients with IBD can have a loss of tolerance to specific bacterial antigens and autoantigens. These distinct antibody response patterns may indicate unique pathophysiological mechanisms in the progression of this complicated disease and may underlie the basis for the development of specific phenotypes (Landers et al., 2002; Peeters et al., 2001).

Numerous serological markers have been proposed as having utility in assessment of IBD patients. The most widely studied markers are the antineutrophil cytoplasmic antibodies (pANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA), particularly for diagnosing IBD and distinguishing CD from ulcerative colitis (Higuchi, 2020; Peppercorn & Kane, 2022a). pANCA is thought to be an antibody corresponding to histone 1 whereas ASCA is an antibody against mannan from baker's yeast (Mitsuyama et al., 2016). Although there have been promising results regarding the clinical validity of these antibodies (Reese et al., 2006; Ruemmele et al., 1998; Sandborn et al., 2000), its utility in indeterminate bowel disease is uncertain (Joossens et al., 2002; Peeters et al., 2001). ASCA were present in 50 percent of patients with celiac disease and described in cystic fibrosis and intestinal tuberculosis, suggesting that they may reflect a nonspecific immune response in small bowel disease (Condino et al., 2005; Granito et al., 2005).

Additional antibody tests under investigation include laminaribioside (ALCA), chitobioside (ACCA), CBir1 flagellin, OmpC, and I2. ALCA and ACCA are antiglycan antibodies whereas the CBir1 flagellin comes from an indigenous species of bacteria (Dotan et al., 2006; Targan et al., 2005). OmpC is an antibody to an outer membrane protein of *E. coli* and I2 is an antibody against the I2 component of *Pseudomonas fluorescens* (Mitsuyama et al., 2016). The accuracy and predictive value of antibody tests is uncertain (Wang et al., 2017) and the prevalence of these antibodies in patients with a variety of inflammatory diseases affecting the gut has not been well-studied.

Additionally, bile acid deficiency--as indicated by serum 7α-hydroxy-4-cholesten-3-one (7C4)--has been documented in patients with irritable bowel syndrome (IBS) (Donato et al., 2018; Vijayvargiya et al., 2018). This test has shown utility as an alternative test to measuring bile acids in stool (Walters & Pattni, 2010), but it is not recommended in the workup for IBD.

Another proposed biomarker for IBD is serum pyruvate kinase M2 (PKM2), which is "emerging" in IBD as a mediator of inflammatory processes. Almousa et al. (2018) evaluated its association with IBD and its correlation with traditional IBD indices, BD disease type, and intestinal microbiota. The authors found that serum PKM2 levels were 6 times higher in IBD patients compared to healthy controls. However, no sensitivity to disease phenotype or localization of inflammation was observed. A positive correlation

between PKM2 and *Bacteroidetes* was identified, as well as a negative correlation between PKM2 and *Actinobacteria*. The investigators concluded that their data "suggests PKM2 as a putative biomarker for IBD and the dysbiosis of microflora in CD," but noted that further validation was required (Almousa et al., 2018).

Genetic studies have identified over 200 distinct susceptibility loci for irritable bowel disease with a significant portion of these overlapping with Crohn's and ulcerative colitis (Jostins et al., 2012; Liu et al., 2015). Most of these are located within introns, which more likely modulate the expression of proteins, with each only conferring a slight increase in risk (Snapper & Abraham, 2020). Altogether, the known loci only explain ~13% of variation in disease liability (Jostins et al., 2012). These results indicate that the genetic architecture of IBD represents that of multifactorial complex traits where a combination of multiple genes, along with the environment, lead to disease (Liu & Anderson, 2014). Given the low predictive value of individual genetic markers and high number of putative risk alleles, genetic testing does not currently offer much in terms of clinical utility (Lichtenstein et al., 2018; Liu & Anderson, 2014; McGovern et al., 2015; Shirts et al., 2012).

Laboratory evidence of inflammation is common in IBD. Fecal calprotectin, lactoferrin, ESR and CRP have each been correlated with disease activity (Lewis, 2011; Menees et al., 2015), but are not specific. Additional inflammatory markers including vascular endothelial growth factor, intercellular adhesion molecule, vascular adhesion molecule, and serum amyloid A offer no significant advantage (Shirts et al., 2012). Fecal calprotectin has been shown to be useful to help differentiate the presence of IBD from irritable bowel syndrome and in monitoring disease activity and response to treatment (Lichtenstein et al., 2018). Inflammation and calprotectin testing are discussed in greater detail in AHS-G2155 and AHS-G2061, respectively.

Clinical Utility and Validity

Panels to improve the predictive value of IBD testing incorporating serologic, genetic, and inflammation markers have been created (Plevy et al., 2013). The clinical validity and utility of antibody tests and panels of combinations of serologic tests for the diagnosis of IBD and the disease course and severity are still uncertain (Benor et al., 2010; Coukos et al., 2012; Kaul et al., 2012; Sura et al., 2014; Wang et al., 2017). For example, Prometheus Biosciences offers a series of tests intended for IBS. This series includes "IBDsgi Diagnostic," which evaluates 17 biomarkers (serological and genetic markers, intended to provide "diagnostic and prognostic clarity,"(Prometheus, 2022a) "Crohn's Prognostic" (evaluates "proprietary serologic (anti-CBir1, anti-OMPC, DNAse sensitive pANCA) and genetic (NOD2 variants SNPs 8,12,13) markers"), and "Monitr" (evaluates 13 biomarkers to provide an "Endoscopic Healing Index Score" which represents endoscopic disease activity) (Prometheus, 2022b). In February 2022, Prometheus announced the release of PredictrPK IFX, a test that helps healthcare providers with biologic dose optimization by using individualized pharmacokinetic modeling. According to the Prometheus site, "PredictrPK IFX combines serology markers, patient-specific variables, current dosing information, and a proprietary machine-learning algorithm to provide individualized actionable insights to optimize the dose and interval for inflammatory bowel disease (IBD) patients treated with infliximab (IFX) or IFX biosimilars" (Prometheus, 2022c).

Mitsuyama et al. (2014) conducted a multicenter study to explore the possible diagnostic utility of antibodies to the CD peptide (ACP) in patients with CD. A total of 196 patients with CD, 210 with UC, 98 with other intestinal conditions, and 183 healthy controls were examined. In CD patients, ACP had a

higher sensitivity and specificity (63.3% and 91.0%, respectively) than ASCA (47.4% and 90.4%, respectively). ACP was also found to be negatively associated with disease duration. The authors concluded that "ACP, a newly proposed serologic marker, was significantly associated with CD and was highly diagnostic. Further investigation is needed across multiple populations of patients and ethnic groups, and more importantly, in prospective studies" (Mitsuyama et al., 2014).

Kaul et al. (2012) performed a meta-analysis/systemic review aimed to evaluate the diagnostic value, as well as the association of anti-glycan biomarkers with IBD susceptible gene variants, disease complications, and the need for surgery in IBD. A total of 23 studies were included consisting of 14 in the review and 9 in the meta-analysis. They found that "individually, anti-Saccharomyces cerevisiae antibodies (ASCA) had the highest diagnostic odds ratio (DOR) for differentiating IBD from healthy (DOR 21.1), and CD from UC (DOR 10.2…)" (Kaul et al., 2012). The authors concluded, "ASCA had the highest diagnostic value among individual anti-glycan markers. While anti-chitobioside carbohydrate antibody (ACCA) had the highest association with complications, ASCA and ACCA associated equally with the need for surgery" (Kaul et al., 2012).

Schoepfer et al. (2008) aimed to determine the accuracy of fecal markers, C-reactive protein (CRP), blood leukocytes, and antibody panels for discriminating IBD from IBS. Sixty-four patients with IBD, 30 patients with IBS, and 42 healthy controls were included within the study. They found that "Overall accuracy of tests for discriminating IBD from IBS: IBD-SCAN 90%, PhiCal Test 89%, LEUKO-TEST 78%, Hexagon-OBTI 74%, CRP 73%, blood leukocytes 63%, CD antibodies (ASCA+/pANCA- or ASCA+/pANCA+) 55%, UC antibodies (pANCA+/ASCA-) 49%. ASCA and pANCA had an accuracy of 78% for detecting CD and 75% for detecting UC, respectively. The overall accuracy of IBD-SCAN and PhiCal Test combined with ASCA/pANCA for discriminating IBD from IBS was 92% and 91%, respectively" (Schoepfer et al., 2008).

Plevy et al. (2013) validated a diagnostic panel incorporating 17 markers. The markers were as follows: "8 serological markers (ASCA-IgA, ASCA-IgG, ANCA, pANCA, OmpC, CBir1, A4-Fla2, and FlaX), 4 genetic markers (ATG16L1, NKX2-3, ECM1, and STAT3), and 5 inflammatory markers (CRP, SAA, ICAM-1, VCAM-1, and VEGF)." A total of 572 patients with CD, 328 with UC, 427 non-IBD controls, and 183 controls were assessed. These results were compared to another panel with serological markers only. The extended panel increased the IBD vs non-IBD discrimination area under the curve from 0.80 to 0.87 and the CD vs UC from 0.78 to 0.93. The authors concluded that "incorporating a combination of serological, genetic, and inflammation markers into a diagnostic algorithm improved the accuracy of identifying IBD and differentiating CD from UC versus using serological markers alone" (Plevy et al., 2013).

Biasci et al. (2019) validated a 17-gene prognostic classifier. The classifier was intended to separate IBD patients into two subgroups of prognosis, IBDhi (poorer prognosis) and IBDlo. Two validation cohorts were used, one of CD (n=66) and one of UC (n=57). IBDhi (separated by the classifier) patients experienced both an "earlier need for treatment escalation (hazard ratio=2.65 (CD), 3.12 (UC)) and more escalations over time (for multiple escalations within 18 months: sensitivity=72.7% (CD), 100% (UC); negative predictive value=90.9% (CD), 100% (UC)" (Biasci et al., 2019).

Czub et al. (2014) compared PKM2 to fecal calprotectin (FC) as markers for mucosal inflammation in IBD. A total of 121 patients (75 with UC, 46 with CD) were compared to 35 healthy controls. The authors found that as a whole, PKM2 was "inferior" to FC. The differences in the area under curve were as follows: 0.10

(FC above PKM2, IBD), 0.14 (UC), and 0.03 (IBD). PKM2 was also considered inferior to FC in differentiating patients from mild UC from healthy patients by an AUC of 0.23 (Czub et al., 2014).

Kovacs et al. (2018) investigated "prognostic potential of classic and novel serologic antibodies regarding unfavorable disease course in a prospective ulcerative colitis (UC) patient cohort". They measured the auto-antibodies anti-neutrophil cytoplasmic (ANCA), anti-DNA-bound-lactoferrin (anti-LFS), anti-goblet cell (anti-GAB) and anti-pancreatic (pancreatic antibody (PAB): anti-CUZD1 and anti-GP2) and the anti-microbial antibodies anti-Saccharomyces cerevisiae (ASCA) IgG/IgA and anti-OMP Plus™ IgA. A total of 187 patients were included. The authors found a total of "73.6%, 62.4% and 11.2% of UC patients were positive for IgA/IgG type of atypical perinuclear-ANCA, anti-LFS and anti-GAB, respectively." Occurrences of PABs were 9.6%, ASCA IgA/IgG was 17.6%, and anti-OMP IgA was 19.8%. IgA type PABs were found to be more prevalent in patients with primary sclerosing cholangitis (37.5% vs. 4.7% for anti-CUZD1 and 12.5% vs. 0% for anti-GP2). IgA type ASCA was associated with a higher risk for requiring long-term immunosuppressant therapy. The authors found that none of the autoantibodies, either alone or in combination, were associated with the "risk of development of extensive disease or colectomy," although "multiple antibody positivity [≥3]" was associated with UC-related hospitalization. Overall, the authors concluded that "Even with low prevalence rates, present study gives further evidence to the role of certain antibodies as markers for distinct phenotype and disease outcome in UC. Considering the result of the multivariate analysis the novel antibodies investigated do not seem to be associated with poor clinical outcome in UC, only a classic antibody, IgA subtype ASCA remained an independent predictor of long-term immunosuppressive therapy" (Kovacs et al., 2018).

Ben-Shachar et al. (2019) evaluated the impact of genotype variations on serological biomarkers. The authors examined three *NOD2* variants (1007fs, G908R, R702W) and an *ATG16L1* variant (A300T). Then, the authors analyzed the antiglycan antibodies anti-*Saccharomyces cerevisiae* (ASCA), antilaminaribioside (ALCA), antichitobioside (ACCA), and antimannobioside carbohydrate (AMCA). A total of 308 IBD patients were included, "130 with Crohn's Disease (CD), 67 with ulcerative colitis (UC), 111 with UC and an ileal pouch (UC-pouch), and 74 healthy controls." ACCA was found to be "positive" in 28% of CD patients with the *ATG16L1* A300T variant, compared to only 3% in patients without the variant. ASCA was found to be positive in 86% of patients with the 1007fs variant, compared to 36% without the variant. UC-pouch patients with the 1007fs variant were also found to have "elevated" ASCA and ALCA levels compared to those without (50% vs 7% and 50% vs 8% respectively). The authors also found that the genetic variants were not associated with serologic responses in healthy controls and "unoperated" UC patients. The authors concluded that "Genetic variants may have disease-specific phenotypic (serotypic) effects. This implies that genetic risk factors may also be disease modifiers" (Ben-Shachar et al., 2019).

Ahmed et al. (2019) examined the association between six serological markers and Crohn's Disease (CD) activity. The six markers evaluated were "ASCA-IgA, ASCA-IgG, anti-OmpC IgA, anti-CBir1 IgG, anti-A4Fla2 IgG and anti-FlaX IgG". A total of 135 patients were included. The authors found that CD patients with high anti-Cbir1 IgG at baseline were 2.06 times more likely to have active clinical disease. The other five autoantibodies were not found to have significant impact on clinical course. The authors concluded that "High levels of anti-Cbir1 IgG appear to be associated with a greater likelihood of active CD. Whether routine baseline testing for anti-Cbir1 IgG to predict a more active clinical course is warranted needs more research" (Ahmed et al., 2020; Duarte-Silva et al., 2019).

Eltabbakh (2021) studied the diagnostic utility of beta 2-microglobulin (B2-M) as a biomarker in patients with IBS and UC. B2-M is a protein released by activated T and B lymphocytes and has shown to increase in inflammatory conditions. 40 patients with UC, 20 patients with IBS, and 20 healthy subjects were enrolled in the study. Overall, there was a higher mean of B2-M values in the UC patients (1.93) than IBS patients (1.51) or healthy subjects (1.43). At a cut off value of >1.5, sensitivity (75%), specificity (70%), PPV (83.3%), NPV (58.3%), and accuracy (0.753%) were measured. It was concluded that "B2-M level may have a diagnostic and differentiating utility between UC cases and IBS-D type as well as a potential indicator of disease activation in UC patients" (Eltabbakh, 2021).

Gao & Zhang (2021) studied the use of serological markers for the diagnosis of Crohn's disease. 196 suspected CD patients were enrolled in the study and ELISA was used to study the expression of various biomarkers including ASCA-IgG, ASCA-IgA, AYMA-IgG, AYCA-IgA, FI2Y-IgG, and pANCA. Overall, ASCA was found to be the most accurate serological marker for the differential diagnosis of CD. It was also noted that a combination of markers resulted in a higher sensitivity and NPV. There was no relation noted between the expression of ASCA and disease behavior at diagnosis (Gao & Zhang, 2021).

Nakov et al. (2022) performed a review of current studies related to IBS and IBD biomarker diagnosis and management, including how to distinguish IBS from IBD (as a note, IBS is a disorder of the gastrointestinal tract while IBD constitutes inflammation or destruction of the bowel wall. Crohn's disease and ulcerative colitis fall under an IBD etiology). The authors focused on the most clinically validated biomarkers to-date and summarized the biological rationale, diagnostic, and clinical value. The authors wrote, "there are well-established serological markers that help differentiate IBS from IBD. These include ASCA, which facilitates the differential diagnosis of Crohn's disease (CD) and ulcerative colitis (UC), predominantly in the disease's early stages. The serum concentration of ASCA is considerably higher in patients with CD than in those with UC. Thus, ASCA can be employed in differentiating organic disease from IBS." They also noted "the other autoantibodies that can be used in distinguishing IBS from IBD are the anti-neutrophil cytoplasmic antibody. They target antigens present in neutrophils and are positive in 50–80% of the UC patients"(Nakov et al., 2022).

Reese et al. (2006) performed a meta-analysis of dozens of studies to assess the diagnostic precision of ASCA and pANCA in pinpointing irritable bowel disease, as well as the role of these particular serum antibodies in differentiating Crohn's from ulcerative colitis. Using 60 different studies, comprising 3,841 UC and 4,019 CD patients, they calculated sensitivity, specificity, and likelihood ratio for different test combinations. The ASCA+ with PANCA- test had the highest sensitivity for Crohn's disease at 54.6%; the specificity was 92.8%. The sensitivity and specificity of pANCA+ tests for ulcerative colitis were 55.3% and 88.5%, respectively. Sensitivity and specificity or pANCA+ were improved in a pediatric subgroup when combined with an ASCA test. In the pediatric cohort, sensitivity was 70.3% and specificity was 93.4%. In conclusion, the authors write that "ASCA and pANCA testing are specific but not sensitive for CD and UC, but that it may be particularly useful for differentiating between CD and UC in the pediatric population" (Reese et al., 2006).

V. Guidelines and Recommendations

American Gastroenterological Association (AGA)

No guideline or position statement from AGA on specific use of immunologic or genetic markers for the diagnosis of inflammatory bowel disease was found. The AGA assessment algorithms used for both

Crohn's disease and ulcerative colitis do not include genetic testing or combinatorial serologic-genetic testing approaches, such as the Prometheus® testing methodology (AGA, 2015).

In 2021, the AGA published a guideline on the medical management of severe luminal and perianal fistulizing Crohn's disease (Feuerstein et al., 2021). While the guideline focuses on therapeutic approaches (i.e., different drug classes for Crohn's disease), it does make a statement on perceived future research needs and evidence gaps. AGA notes: "There remains an urgent need for improved patient-specific predictors, clinical and biologic, of response and harm to a particular drug or drug class to improve the rational choice of initial and second-line therapeutic agents in a given patient. The need is especially great in special populations, such as those with fistulizing disease or aggressive and recurrent fibrostenosing disease. Overall, the data on risk-stratifying individual patients into low and high risk of disease complications and disability remain poor" (Feuerstein et al., 2021).

American College of Gastroenterology (ACG)

The ACG published guidelines (Lichtenstein et al., 2018) on the management of Crohn's disease which state:

- "The diagnosis of Crohn's disease (CD) is based on a combination of clinical presentation and endoscopic, radiologic, histologic, and pathologic findings that demonstrate some degree of focal, asymmetric, and transmural granulomatous inflammation of the luminal GI tract. Laboratory testing is complementary in assessing disease severity and complications of disease. There is no single laboratory test that can make an unequivocal diagnosis of CD. The sequence of testing is dependent on presenting clinical features."
- "Initial laboratory investigation should include evaluation for inflammation, anemia, dehydration, and malnutrition."
- "Genetic testing is not indicated to establish the diagnosis of Crohn's disease."
- "Routine use of serologic markers of IBD to establish the diagnosis of Crohn's disease is not indicated."

The ACG guidelines on Ulcerative Colitis in adults (Rubin et al., 2019) state:

- "We recommend against serologic antibody testing to establish or rule out a diagnosis of UC (strong recommendation, very low quality of evidence)."
- "We recommend against serologic antibody testing to determine the prognosis of UC (strong recommendation, very low quality of evidence)."
- The ACG also mentions perinuclear antineutrophil cytoplasmic antibodies (pANCAs) as a proposed serological marker, but they observe that "there is currently no role for such testing to determine the likelihood of disease evolution and prognosis" and that the marker has low sensitivity for diagnostic purposes.
- Overall, "the yield of genetic or serologic markers in predicting severity and course of UC has been modest at best, and their use cannot be recommended in routine clinical practice based on available data (Rubin et al., 2019)."

European Crohn's and Colitis Organisation (ECCO)

ECCO states that the Montréal classification of CD is advocated. Therefore, "genetic tests or serological markers should currently not be used to classify CD in clinical practice" (Gomollón et al., 2016).

In a 2017 update for UC, ECCO states that "the routine clinical use of genetic or serological molecular markers is not recommended for the classification of ulcerative colitis." ECCO also notes that the most widely studied marker is the pANCAs, but they have "limited sensitivity" and "their routine use for the diagnosis of UC and for therapeutic decisions is not clinically justified" (Magro et al., 2017).

ECCO also published a "harmonization of the approach to Ulcerative Colitis Histopathology". A section titled "Correlation of Histological Scores with Biomarkers" is included. However, only fecal biomarkers (such as fecal lactoferrin and calprotectin) are mentioned, with no mention of serological biomarkers (F. Magro et al., 2020).

ECCO also published the "ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment." While the guideline mainly focused on therapeutic agents, it does advocate for identification of important biomarkers to biologic effect. ECCO writes, "there is a clear need to identify biomarkers that could guide therapeutic choices, and to conduct appropriately sized head-to-head trials that could allow for the identification of patient subgroups who would benefit from a given biologic over the other" (Torres et al., 2019).

World Gastroenterology Organisation (WGO)

Concerning the use of p-ANCA and ASCA to diagnose UC and CD, the WGO states, "These tests are unnecessary as screening tests, particularly if endoscopy or imaging is going to be pursued for more definitive diagnoses. p-ANCA may be positive in Crohn's colitis and hence may not be capable of distinguishing CD from UC in otherwise unclassified colitis. ASCA is more specific for CD. These tests may have added value when there may be subtly abnormal findings, but a definitive diagnosis of inflammatory bowel disease is lacking. They may also be helpful if considering more advanced endoscopic techniques such as capsule endoscopy or double-balloon endoscopy, such that a positive ASCA test may provide stronger reasons for evaluating the small bowel." Later, the WGO also notes, "There are several other antibody tests, mostly for microbial antigens, that increase the likelihood of CD either singly, in combination, or as a sum score of the ELISA results for a cluster of antibodies. These tests are costly and not widely available. The presence of these antibodies, including a positive ASCA, would increase the likelihood that an unclassified IBD-like case represents Crohn's disease (Bernstein et al., 2016)."

Working Group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America

A clinical report (Bousvaros et al., 2007) noted that:

"A positive ANCA does not differentiate between UC and Crohn colitis."

"Genetic testing cannot as yet reliably differentiate UC from CD of the colon."

The Working Group also observed that in the largest study of prospective markers for UC, the majority of patients remained seronegative for both ASCA and ANCA.

North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN)

NASPGHAN published a guideline regarding the management of patients with "Very Early-Onset Inflammatory Bowel Disease (VEO-IBD)". This guideline defines this cohort as a patient of the pediatric IBD population presenting at under 6 years of age. The guideline makes the following remarks on evaluation of IBD in this population:

- "…genetic sequencing is often necessary to identify the specific monogenic forms of VEO-IBD, or to confirm a suspected defect."
- "Targeted panels should be performed first in cases of infantile onset IBD, when the phenotype is consistent with a known defect, history of consanguinity, and abnormal immunology studies."
- "Currently, WES is most often performed in the setting of a negative targeted panel, however, there are select cases in which WES may be indicated instead of a targeted panel, such as those patients who present with a phenotype that is not previously described."
- "At this time, WGS should be reserved for cases in which WES is negative, yet there remains a high suspicion of a monogenic defect given the young age of onset, disease severity, family history, and complex phenotype including associated autoimmunity."
- "In general, the gene defects that have been detected with the highest frequency in patients with VEO-IBD can prompt specific targeted therapies that include: defects that lead to CGD (NADPH complex defects), IL-10R and XIAP." (Kelsen et al., 2019)

National Institute for Health and Care Excellence (NICE)

NICE does not mention any serological or genetic biomarkers in its reviews of management of UC or CD (NICE, 2019a, 2019b).

British Society of Gastroenterology (BSG)

The BSG published guidelines on the "management of inflammatory bowel disease [IBD] in adults". In it, they made the following comments regarding use of biomarkers in IBD:

- "…more evidence is also needed of the role of faecal calprotectin or other biomarkers as non-invasive surrogates for mucosal healing."
- "Further studies are required to evaluate the use of drug levels and biomarkers to determine personalized dosing for patients."
- "If a response [to treatment] is unclear, then measurement of biomarkers, serum C-reactive protein and faecal calprotectin, or comparison of disease activity scores or PROMs with baseline values, may be helpful."
- "We suggest that genetic testing for monogenic disorders should be considered in adolescents and young adults who have had early onset (before 5 years of age) or particularly aggressive, refractory or unusual IBD presentations (GRADE: weak recommendation, very low-quality evidence." (Lamb et al., 2019)

European Crohn's and Colitis Organisation (ECCO) and the European Society of Gastrointestinal and Abdominal Radiology (ESGAR)

- These joint guidelines include some relevant items on inflammatory bowel disease (IBD), which includes both Crohn's disease (CD) and ulcerative colitis (UC). These items include:
- "A single reference standard for the diagnosis of Crohn's disease [CD] or ulcerative colitis [UC] does not exist. The diagnosis of CD or UC is based on a combination of clinical, biochemical, stool, endoscopic, cross-sectional imaging, and histological investigations."
- "Genetic or serological testing is currently not recommended for routine diagnosis of CD or UC."
- "On diagnosis, complementary investigations should focus on markers of disease activity, malnutrition, or malabsorption."
- "Serological markers may be used to support a diagnosis, though the accuracy of the best available tests [pANCA and ASCAs] is rather limited and hence ineffective at differentiating colonic CD from UC. Similarly, the additional diagnostic value of antiglycan and antimicrobial antibodies, such as anti-OmpC and CBir1, is small." (Maaser et al., 2018)

European Crohn's and Colitis Organisation (ECCO) and European Society of Pediatric Gastroenterology, Hepatology and Nutrition (European Society for Paediatric Gastroenterology Hepatology and Nutrition)

This joint guideline was published regarding "Management of Paediatric Ulcerative Colitis" Although there was no mention of serological markers, the guideline did make this comment on "very early-onset inflammatory bowel disease presenting as colitis", which is as follows:

- "Unusual disease evolution, history of recurrent infections, HLH [hemophagocytic lymphocytic histiocytosis], and non-response to multiple IBD medications may indicate an underlying genetic defect which should prompt genetic and/or immunological analyses at any age during childhood." (Turner et al., 2018)

World Society of Emergency Surgery and the American Association for the Surgery of Trauma

WSES and AAST released joint guidelines on the management of inflammatory bowel disease in the emergency setting. When assessing an acute abdomen in patients with IBD, "laboratory tests including full blood count, electrolytes, liver enzymes, inflammatory biomarkers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and serum albumin and pre-albumin (to assess nutritional status and degree of inflammation) are mandatory" (De Simone et al., 2021).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82397	Chemiluminescent assay
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
86021	Antibody identification; leukocyte antibodies
86255	Fluorescent noninfectious agent antibody; screen, each antibody
86671	Antibody; fungus, not elsewhere specified
88346	Immunofluorescence, per specimen; initial single antibody stain procedure
88350	Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
0164U	Gastroenterology (irritable bowel syndrome [IBS]), immunoassay for anti-CdtB and anti-vinculin antibodies, utilizing plasma, algorithm for elevated or not elevated qualitative results Proprietary test: ibs-smart™ Lab/Manufacturer: Gemelli Biotech
0176U	Cytolethal distending toxin B (CdtB) and vinculin IgG antibodies by immunoassay (i.e., ELISA) Proprietary test: IBSchek® Lab/Manufacturer: Commonwealth Diagnostics International, Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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Lyme Disease Testing

Policy #: AHS – G2143	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/26/22, 10/17/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Lyme disease is a common multisystem inflammatory disease caused by spirochetes of the family *Borreliaceae* transmitted through the bite of an infected tick of the genus Ixodes (Barbour, 2022). Lyme disease affects the skin in its early, localized stage, and spreads to the joints, nervous system, and other organ systems in its later, disseminated stages (Hu, 2022).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For individuals with symptoms of Lyme disease and a history of travel to a region endemic for Lyme (with or without a history of a tick bite), serologic testing (2-tier testing strategy using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay or FDA-cleared second EIA assay) **MEETS COVERAGE CRITERIA**.
2. For individuals with a history of travel to a region endemic for Lyme, serologic testing (2-tier testing strategy using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay or FDA-cleared second EIA assay) **MEETS COVERAGE CRITERIA in** any of the following situations:
   a. For individuals with acute myocarditis/pericarditis of unknown cause.

b. For individuals with meningitis, encephalitis, or myelitis.

c. For individuals with painful radiculoneuritis.

d. For individuals with mononeuropathy multiplex including confluent mononeuropathy multiplex.

e. For individuals with acute cranial neuropathy.

3. Serologic testing **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:

f. For individuals with an erythema migrans (EM) rash (patients with skin rashes consistent with EM who reside in or who have recently traveled to an endemic area should be treated for Lyme disease).

g. To screen asymptomatic patients living in endemic areas.

h. For individuals with non-specific symptoms only (e.g., fatigue, myalgias/arthralgias).

i. For individuals with amyotrophic lateral sclerosis.

j. For individuals with relapsing-remitting multiple sclerosis.

k. For individuals with Parkinson's disease.

l. For individuals with dementia or cognitive decline, or new-onset seizures.

m. For individuals with psychiatric illness.

4. Detection of *Borrelia burgdorferi* by nucleic acid identification techniques (direct **or** amplified probe) **DOES NOT MEET COVERAGE CRITERIA**.

5. For individuals who have previously tested positive for Lyme disease, repeat serologic testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

6. All other testing for *Borrelia burgdorferi* not described above **DOES NOT MEET COVERAGE CRITERIA**.

7. For the diagnosis of Lyme disease, testing of the individual tick **DOES NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Lyme disease can be caused by several species in the spirochete family *Borreliaceae*; however, infection in North America is predominately caused by *B. burgdorferi*. Much less commonly, in the upper midwestern United States, cases have been associated with B. *mayonii* (Mead & Schwartz, 2022; Pritt et al., 2016). The taxonomic classification system for this species is undergoing revision, and the genus name may be represented as either Borrelia or *Borreliella* (Adeolu & Gupta, 2014; Margos et al., 2017). Borrelia burgdorferi occurs naturally in reservoir hosts, including small mammals and birds (Hyde, 2017). Ixodes scapularis and I. *pacificus* become infected with *B. burgdorferi* while feeding on the blood of natural reservoir hosts. Transmission to humans results from the bite of an infected tick (Bacon et al., 2008). Spirochete transmission times and virulence depend upon the tick and Borrelia species, and

infection can never be excluded after a tick bite irrespective of the estimated duration of attachment time (Cook, 2015).

In the earliest stage of Lyme disease, *B. burgdorferi* disseminates from the site of the tick bite resulting in the colonization of dermal tissue and localized infection characterized by a painless bulls-eye rash called erythema migrans, experienced by approximately 70–80% of patients at the site of the tick bite. This is accompanied by non-specific flu-like symptoms, including headache, neck stiffness, malaise, fatigue, myalgia, and fever. During localized infection, the number of *B. burgdorferi* cells increases in the dermal tissue. If left untreated, *B. burgdorferi* can disseminate from the site of the tick bite through the bloodstream and/or lymphatic system to invade and colonize various tissues days to weeks after infection. This can affect the heart, joints, and nervous system. Months to years after exposure to *B. burgdorferi*, affected individuals can experience different manifestations, including neuroborreliosis, Lyme carditis, and/or arthritis (Hyde, 2017).

The CDC reports that about 476,000 Americans are diagnosed with Lyme disease each year, but they estimate that only about 300,000 people get Lyme disease each year. The CDC notes that these numbers likely differ because the 476,000 people treated for Lyme disease, and patients are often treated presumptively and without proper testing (CDC, 2021b).

Even following antibiotic treatment, a subset of patients continues to present with arthritic symptoms; this has been designated as postinfectious, antibiotic-refractory Lyme arthritis (Hyde, 2017). The term "post-Lyme disease syndrome" (PTLDS) is often used to describe the nonspecific symptoms (such as headache, fatigue, and arthralgias) that may persist for months after treatment of Lyme disease. For the majority of patients, these symptoms improve gradually over six months to one year (Hu, 2022). Weitzner et al. (2015) found that "PTLDS may persist for [over] 10 years in some patients with culture-confirmed early Lyme disease. Such long-standing symptoms were not associated with functional impairment or a particular strain of B. *burgdorferi*."

The diagnosis of Lyme disease is based on an individual's history of possible exposure to ticks, the presence of characteristic signs and symptoms, and blood test results (Hu, 2022). Direct detection of *Borrelia burgdorferi* has limited applications (Marques, 2015). Thus, most laboratory confirmation of Lyme disease involves the detection of antibody responses against *B. burgdorferi* in serum (Schriefer, 2015). Serology testing is not recommended for patients who do not have symptoms typical of Lyme disease (Marques, 2015), as current assays do not distinguish between active and past infection, thus a positive result is more likely to be a false positive. Early diagnosis of erythema migrants should be made without testing because the lesion appears prior to development of a diagnostic, adaptive immune response (Hu, 2022).

Serological testing using the two-tier algorithm, comprising a first screening enzymatic immunoassay (EIA), followed by a confirmatory Western blot test, is the gold standard for Lyme disease diagnoses (Bunikis & Barbour, 2002; Hu, 2022; John & Taege, 2019). Standardized 2-tier testing (STTT) is the recommended diagnostic technique for Lyme disease in clinical practice (CDC, 2021a). Although STTT detection of early, localized infection is poor, STTT detection of late disease is excellent (Schriefer, 2015). Evidence of seronegative late Lyme disease is unconvincing (Halperin, 2015). A systematic review

has shown that the sensitivity of serology for Lyme disease in early localized infection is 50%, but the algorithm performs well in late stages of the infection, where the sensitivity approaches 100% (Waddell et al., 2016).

On July 29, 2019, the FDA approved several Lyme disease serologic assays, including ZEUS ELISA, allowing for an EIA rather than Western blot as the second test in the two-tier algorithm (CDC, 2019). ZEUS ELISA is a Modified Two-Tiered Testing (MTTT) Algorithm that replaces the second-tier Western blot with a more sensitive and specific methodology, such as ELISA. According to ZEUS Scientific, MTTT reduces the number of missed clinically positive patient samples and improves lab efficiency (ZEUS_Scientific, 2019). Compared to the traditional STTT, the MTTT algorithms improve sensitivity to detect early infections and have equivalent sensitivity for detecting late-stage infections and comparable specificity. In addition, MTTT may have the benefit of improved sensitivity in identifying positive cases in patients infected with related strains of *Borrelia*. In a study by Davis, one case of infection with a European genospecies of Borrelia was detected by MTTT, which was missed by STTT (Davis et al., 2020). The Canada Communicable Disease Report (CCDR) agrees with the FDA recommendation, advising that "Diagnostic improvements in sensitivity of [Lyme disease] testing without significant loss of specificity have been consistently reported when MTTT is compared with STTT in studies conducted in highly [Lyme disease] endemic regions" (CCDR, 2020).

Polymerase chain reaction (PCR) testing may be useful in the early stages of a Lyme disease infection before an immune response occurs and is also helpful when testing for reinfection. Other potential techniques for Lyme disease diagnostics include cell culture, ELISA, urine testing, and multiplex testing techniques (John & Taege, 2019).

Proprietary Testing

Waddell et al. (2016) assessed the accuracy of the traditional diagnostic tests of Lyme disease. A total of 11 studies with 34 lines of data were evaluated for the overall accuracy. The overall sensitivity was found to be 82%, and the overall specificity was found to be 94.2%. Fifteen studies were examined for Stage 1 of Lyme disease, and the sensitivity was found to be 54%; however, the specificity was calculated to be 96.8%. Stage 2 (5 studies, 6 lines) had a sensitivity of 79.1% and specificity of 97.7%, and Stage 3 (9 studies, 20 lines) had a sensitivity of 94.7% and specificity of 96.1%. The CDC immunoblots (second tier, 2 studies, 4 lines) were estimated at 91% sensitivity and 99% specificity (Waddell et al., 2016).

Other diagnostic tests have been created but not widely validated (Hu, 2022). For instance, Wormser et al. (2013) evaluated a C6 enzyme-linked immunosorbent assay (ELISA) as a single-step, serodiagnostic test that uses a reference standard of two-tier testing. This test provided increased sensitivity in early Lyme disease with comparable sensitivity in later manifestations of the disease. Four hundred and three samples were compared to the sensitivities of the traditional two-tier tests, and the C6 ELISA was measured to have a 66.5% sensitivity and a 35.2% sensitivity, both of which were more sensitive than the individual steps of the STTT approach. The specificity was evaluated with over 2200 blood donors, and the C6 ELISA was evaluated at 98.9% specificity (Wormser et al., 2013).

Urine testing for diagnosis of Lyme disease is also available from multiple laboratories. For example, Igenex (2017b) claims that the urine tests "are useful during the acute phase of infection before antibodies are present, in seronegative patients, in patients with vague symptoms of long duration, and previously-treated patients with recurring symptoms." However, the American Academy of Pediatrics (AAP) states that urine tests for *B. burgdorferi* "have been found to be invalid on the basis of independent testing or to be too nonspecific to exclude false-positives (AAP, 2018)." The CDC also includes urine testing for Lyme disease within their list of laboratory tests that are not recommended (CDC, 2018).

Igenex's proprietary Immunoblot has been used to detect IgM and IgG antibodies to diagnose Lyme disease. From the sample report, Igenex has stated that "Recombinant *B. burgdorferi* species antigens are sprayed at specific positions onto a nitrocellulose membrane and cut into strips. These strips are used to detect *B. burgdorferi* specific antibodies in patient serum (Igenex, 2017b)." Eight total species of *Borrelia* are detected by this test; based on 174 samples, the ImmunoBlot was found to have a sensitivity of 90.9% and specificities of 98% (IgM) and 98.7% (IgG) (Igenex, 2017b). Igenex also has a PCR-based test for the detection of *B. burgdorferi*. Four hundred and two positive samples for *B. burgdorferi* were evaluated based on Igenex's proprietary PCR test and the CDC diagnostic criteria (the traditional two-tiered test). Out of the 402 samples, 236 were considered positive by the proprietary PCR test and 70 were considered positive per the CDC criteria (Igenex, 2017a).

Clinical Utility and Validity

Waddell et al. (2016) assessed the accuracy of the traditional diagnostic tests of Lyme disease. A total of 11 studies with 34 lines of data were evaluated for the overall accuracy. The overall sensitivity was found to be 82%, and the overall specificity was found to be 94.2%. Fifteen studies were examined for Stage 1 of Lyme disease, and the sensitivity was found to be 54%; however, the specificity was calculated to be 96.8%. Stage 2 (five studies, six lines) had a sensitivity of 79.1% and specificity of 97.7%, and Stage 3 (nine studies, 20 lines) had a sensitivity of 94.7% and specificity of 96.1%. The CDC immunoblots (second tier, two studies, four lines) were estimated at 91% sensitivity and 99% specificity (Waddell et al., 2016).

Joung et al. (2019) note that while the CDC recommends serological methods for Lyme disease testing, it is expensive (over $400/test) and can take longer than 24 hours to obtain results; therefore, a cost-effective and rapid assay was developed to address these challenges. This assay can detect early-stage Lyme disease and "assays for antibodies specific to seven *Borrelia* antigens and a synthetic peptide in a paper-based multiplexed vertical flow assay (xVFA)"; the specificity of this test was identified at 87% and sensitivity at 90.5% (Joung et al., 2019).

Shakir et al. (2019) used a total of 379 whole blood samples to evaluate ChromaCode's Research Use Only (RUO) nine target High-Definition PCR (HDPCR™) Tick-Borne Pathogen (TBP) panel. Results were compared to clinically validated real-time PCR assays and laboratory developed tests. The final positive percent agreement and negative percent agreement "for the TBP panel was 97.7% (95% CI 95.2% - 99.0%) and 99.6% (95% CI 99.3% - 99.8%), respectively, with an overall agreement of 99.5% (95% CI 99.2% - 99.7%)" with the laboratory developed tests" (Shakir et al., 2019).

Nigrovic et al. (2019) evaluated the Lyme disease PCR test compared to the traditional two-tier assessment method (a positive or equivocal EIA and a positive immunoblot test). In total, 124 were tested and 54 had Lyme disease. However, only 23 of the Lyme disease patients had a positive PCR test, giving a sensitivity of 41.8% and specificity of 100% (Nigrovic et al., 2019). These results show that the Lyme disease PCR test has low sensitivity.

Davis et al. (2020) evaluated the effectiveness of the MTTT algorithm compared to the STTT algorithm. Modified two-tiered testing (MTTT) algorithm uses a second enzyme immunoassay (EIA) instead of the immunoblots for samples that test positive or equivocal on the first EIA. Retrospective chart reviews were performed on 10,253 specimens tested for Lyme disease (LD) serology. "Patients were classified as having Lyme disease if they had a positive STTT result, a negative STTT result but symptoms consistent with Lyme disease, or evidence of seroconversion on paired specimens (Davis et al., 2020)." Of the 10,253 specimens, 9,806 (95.6%) were negative for Lyme disease and 447 patients tested positive. Of the 447 patients, 227 were classified as patients with Lyme disease. "Of the 227 patients classified as having LD, 65 (28.6%) had early localized infections, 67 (29.5%) had early disseminated infections, 26 (11.5%) had late LD, 61 (26.9%) had evidence of old infections, and 8 (3.5%) had posttreatment LD syndrome. Of the remaining 63 patients with early localized disease, 16 (25.4%) were positive by MTTT but negative by STTT. The MTTT identified an additional four (6.6%) cases of early disseminated infection and one case (3.8%) in late LD (Davis et al., 2020)." Overall, MTTT identified additional cases in early localized and early disseminated infections and detected 25% more early infections with a specificity of 99.56% (99.41 to 99.68%) compared to the STTT.

van Gorkom et al. (2020) evaluated the utility of an in-house and a commercial enzyme-linked immunosorbent spot (ELISpot) assay for the diagnosis of Lyme neuroborreliosis (LNB). Peripheral blood mononuclear cells (PBMCs) were isolated from eighty-seven patients diagnosed with LNB at Diakonessenhuis Hospital, Utrecht, and the St Antonius Hospital, Nieuwegein, the Netherlands between March 2014 and November 2017. In-house *Borrelia* ELISpot assay and the commercial LymeSpot assay. However, it was found that both tests performed unsatisfactorily—the sensitivity for the *Borrelia* ELISpot yielded a sensitivity of 61.1% (95% CI: 38.9-77.8%) and a specificity of 66.7(42.0-81.2%), while the LymeSpot assay produced 66.7% (95% CI: 44.4-88.9%) and 59.4% (95% 44.9-72.5%), respectively. Moreover, low PPVs for ELISpot and LymeSpot were observed (30.6% vs. 29.7%, respectively), further corroborate their poor diagnostic performance. The researchers do acknowledge a few shortcomings in their study, namely that the isolation procedure for the PBMC deviated from that of the LymeSpot assay—however, the deviations from protocol were allowed for the technician to minimize differences when comparing across assays to allow for fairer comparison of results. Though this was the case, they believe still that the deviations "from the recommended protocol are not critical", and as such they uphold "the conclusion stands that both ELISpot assays cannot help to diagnose active LNB" (van Gorkom et al., 2020).

Sabin et al. (2023) compared the MTTT algorithm to the STTT. The authors compared samples from 320 patients. "The MTTT confirmed the illness in 116 subjects (36%, P = 0.007), and 30 (26%) were negative by the STTT." MTTT sensitivity was increased in early infection, but insufficiently sensitive to non-*Borrelia* species infections. The authors concluded that "Routine adoption of MTTT would improve sensitivity for early Lyme disease attributable to B. burgdorferi, but may not capture illness attributed to B. mayonii and B. miyamotoi" (Sabin et al., 2023).

IV. Guidelines and Recommendations

The Centers for Disease Control and Prevention (CDC)

The CDC currently recommends a two-step process when testing blood for evidence of antibodies against the Lyme disease bacteria. Both steps can be done using the same blood sample.

- **The first step** uses a testing procedure called "EIA" (enzyme immunoassay) or rarely, an "IFA" (indirect immunofluorescence assay).
- **If this first step is negative**, no further testing of the specimen is recommended.
- **If the first step is positive** or indeterminate (sometimes called "equivocal"), the second step should be performed.
- **The second step** uses a test called an immunoblot test, commonly, a "Western blot" test.
- Results are considered positive only if the EIA/IFA and the immunoblot are both positive (CDC, 2021a; Mead et al., 2019).

CDC Guidelines on Non-Recommended Lab Tests:

Some laboratories offer Lyme disease testing using assays whose accuracy and clinical usefulness have not been adequately established. Examples of unvalidated tests include:

1. Capture assays for antigens in urine
2. Culture, immunofluorescence staining, or cell sorting of cell wall-deficient or cystic forms of *B. burgdorferi*
3. Lymphocyte transformation tests
4. Quantitative CD57 lymphocyte assays
5. "Reverse Western blots"
6. In-house criteria for interpretation of immunoblots
7. Measurements of antibodies in joint fluid (synovial fluid)
8. IgM or IgG tests without a previous ELISA/EIA/IFA (CDC, 2018b)

In the 2019 update concerning the CDC recommendations for serologic diagnosis of Lyme disease, they state, "When cleared by FDA for this purpose, serologic assays that utilize EIA rather than western immunoblot assay in a two-test format are acceptable alternatives for the laboratory diagnosis of Lyme disease. Based on the criteria established at the 1994 Second National Conference on Serologic Diagnosis of Lyme Disease, clinicians and laboratories should consider serologic tests cleared by FDA as CDC-recommended procedures for Lyme disease serodiagnosis (Mead et al., 2019)."

The Infectious Diseases Society of America (IDSA), The American Academy of Neurology (AAN), and The American College of Rheumatology (ACR)

The IDSA, AAN and ACR have published clinical practice guidelines for the prevention, diagnosis, and treatment of Lyme disease. The guidelines include the following statements:

Lyme Disease Testing, continued

- Following a tick bite, "We recommend submitting the removed tick for species identification. (good practice statement)
- We recommend against testing a removed Ixodes tick for *B. burgdorferi* (strong recommendation, moderate quality evidence). The presence or absence of *B. burgdorferi* in an Ixodes tick removed from a person does not reliably predict the likelihood of clinical infection.
- We recommend against testing asymptomatic patients for exposure to *B. burgdorferi* following an Ixodes spp. tick bite (strong recommendation, moderate-quality evidence).
- In patients with potential tick exposure in a Lyme disease endemic area who have 1 or more skin lesions compatible with erythema migrans, we recommend clinical diagnosis rather than laboratory testing (strong recommendation, moderate quality evidence).
- In patients with 1 or more skin lesions suggestive of, but atypical for erythema migrans, we suggest antibody testing performed on an acute-phase serum sample (followed by a convalescent-phase serum sample if the initial result is negative) rather than currently available direct detection methods such as polymerase chain reaction (PCR) or culture performed on blood or skin samples (weak recommendation, low-quality evidence). Comment: If needed, the convalescent-phase serum sample should be collected at least 2–3 weeks after collection of the acute-phase serum sample.
- When assessing patients for possible Lyme neuroborreliosis involving either the peripheral nervous system (PNS) or central nervous system (CNS), we recommend serum antibody testing rather than PCR or culture of either cerebrospinal fluid (CSF) or serum (strong recommendation, moderate-quality evidence).
- If CSF testing is performed in patients with suspected Lyme neuroborreliosis involving the CNS, we (a) recommend obtaining simultaneous samples of CSF and serum for determination of the CSF: serum antibody index, carried out by a laboratory using validated methodology, (b) recommend against CSF serology without measurement of the CSF: serum antibody index, and (c) recommend against routine PCR or culture of CSF or serum (strong recommendation, moderate-quality evidence).
- In patients presenting with 1 or more of the following acute disorders: meningitis, painful radiculoneuritis, mononeuropathy multiplex including confluent mononeuropathy multiplex, acute cranial neuropathies (particularly VII, VIII, less commonly III, V, VI, and others), or in patients with evidence of spinal cord (or rarely brain) inflammation, the former particularly in association with painful radiculitis involving related spinal cord segments, and with epidemiologically plausible exposure to ticks infected with *B. burgdorferi*, we recommend testing for Lyme disease (strong recommendation, moderate-quality evidence).
- In patients with typical amyotrophic lateral sclerosis, relapsing-remitting multiple sclerosis, Parkinson's disease, dementia or cognitive decline, or new-onset seizures, we recommend against routine testing for Lyme disease (strong recommendation, low-quality evidence).
- In patients with neurological syndromes other than those listed… in the absence of a history of other clinical or epidemiologic support for the diagnosis of Lyme disease, we recommend against screening for Lyme disease (strong recommendation, low-quality evidence).

Lyme Disease Testing, continued

- In patients presenting with nonspecific magnetic resonance imaging white matter abnormalities confined to the brain in the absence of a history of other clinical or epidemiologic support for the diagnosis of Lyme disease, we suggest against testing for Lyme disease (weak recommendation, low-quality evidence).
- In patients with psychiatric illness, we recommend against routine testing for Lyme disease (strong recommendation, low-quality evidence).
- In children presenting with developmental, behavioral, or psychiatric disorders, we suggest against routinely testing for Lyme disease (weak recommendation, low-quality evidence).
- In patients with acute myocarditis/pericarditis of unknown cause in an appropriate epidemiologic setting, we recommend testing for Lyme disease (strong recommendation, low-quality evidence)
- In patients with chronic cardiomyopathy of unknown cause, we suggest against routine testing for Lyme disease (weak recommendation, low-quality evidence)
- When assessing for possible Lyme arthritis, we recommend serum antibody testing over PCR or culture of blood or synovial fluid/tissue (strong recommendation, moderate quality of evidence)
- In seropositive patients for whom the diagnosis of Lyme arthritis is being considered but treatment decisions require more definitive information, we recommend PCR applied to synovial fluid or tissue rather than *Borrelia* culture of those samples (strong recommendation, moderate quality of evidence) (Lantos et al., 2021)."

The guideline also made several relevant comments on the above recommendations:

- The guideline commented that knowing tick characteristics (such as "species, life stage, and an assessment of the degree of blood engorgement") is helpful for early guidance, such as antibiotic management.
- "Serologic testing of asymptomatic patients following a tick bite does not help with treatment decisions."
- "Association of Lyme disease with meningitis, cranial neuritis, radiculoneuritis, and other forms of mononeuropathy multiplex is well established…The few systematic studies that have been performed have failed to identify consistent associations between Lyme disease and amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, or Parkinson's disease…These recommendations place a high value on avoiding false positive Lyme disease test results, which can delay appropriate medical evaluation and treatment of other disorders and lead to unnecessary antibiotic exposure and potential side effects."
- "The main disadvantage of this approach [the traditional 'two-tiered approach' is that seroreactivity after successfully treated Lyme borreliosis may persist for years, complicating test interpretation in patients with known previous exposure and/or in patients from highly endemic areas where background seroprevalence is substantial. In such patients, after seroreactivity has been demonstrated, synovial fluid or synovial tissue *B. burgdorferi* PCR may improve diagnostic specificity."

The American College of Rheumatology (ACR)

The ACR also recommends that "the musculoskeletal manifestations of Lyme disease include brief attacks of arthralgia or intermittent or persistent episodes of arthritis in one or a few large joints at a time, especially the knee. Lyme testing in the absence of these features increases the likelihood of false positive results and may lead to unnecessary follow-up and therapy. Diffuse arthralgias, myalgias or fibromyalgia alone are not criteria for musculoskeletal Lyme disease (ACR, 2013)."

Committee on Infectious Diseases, American Academy of Pediatrics, 31st Edition

The Committee on Infectious Diseases released joint guidelines with the American Academy of Pediatrics. They state that the standard testing method for Lyme disease is the two-tier testing algorithm. The initial test is an ELISA or EIA or an immunofluorescent antibody test (IFA) followed by a Western immunoblot. Only specimens that test positive or equivocal on the first test need to be tested with the immunoblot. (AAP, 2018)

The Red Book states that no PCR tests for *B. burgdorferi* are FDA-approved and are not routinely recommended, although PCR testing of joint fluid from a patient with Lyme arthritis may establish a diagnosis. Other tests, such as urine tests for *B. burgdorferi,* the CD57 assay, novel culture techniques, and antibody panels are considered "invalid" as they are not accurate enough to exclude false positive results. The Red Book also notes that the specificity of the C6 EIA does not exceed the specificity of immunoblot (AAP, 2018).

National Institute for Health and Care Excellence (NICE)

NICE recommends diagnosis without laboratory testing in patients with erythema migrans. For patients without erythema migrans, NICE states to consider using an ELISA test. If this ELISA is positive or equivocal, then an immunoblot may be performed. If both tests are positive, then Lyme disease may be diagnosed (NICE, 2018).

NICE also published guidelines in 2019 with the following recommendations:

- "People presenting with erythema migrans are diagnosed and treated for Lyme disease based on clinical assessment, without laboratory testing.
- People with suspected Lyme disease without erythema migrans who have a negative enzyme-linked immunosorbent assay (ELISA) test carried out within 4 weeks of their symptoms starting may have the test repeated 4 to 6 weeks later if Lyme disease is still suspected (NICE, 2019)."

NICE also produced a diagnostic algorithm with the following recommendations:

- "If Lyme disease is still suspected in people with a negative ELISA who have had symptoms for 12 weeks or more, perform an immunoblot test.
- Carry out an immunoblot test, despite an initial negative ELISA, when there is clinical suspicion of Lyme disease. Diagnose Lyme disease in people with symptoms of Lyme disease and a positive immunoblot test.
- If the immunoblot test for Lyme disease is negative (regardless of the ELISA result) but symptoms persist, consider a discussion with or referral to a specialist, to: review whether further tests may be

needed for suspected Lyme disease, for example, synovial fluid aspirate or biopsy, or lumbar puncture for cerebrospinal fluid analysis or consider alternative diagnoses (both infectious, including other tick-borne diseases, and non-infectious).

- Initial testing with a combination IgM and IgG ELISA for Lyme disease should be offered because the evidence generally showed better accuracy (both sensitivity and specificity) for combined tests compared to IgM-only and IgG-only tests. The evidence was best for tests based on purified or recombinant antigens derived from the VlsE protein or its IR6 domain peptide (such as a C6)."

This diagnostic algorithm was primarily based off of NICE's 2018 guidelines (NICE, 2018).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

A search for "Lyme disease" on the FDA website on January 06, 2021 yielded 16 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86617	Antibody; *Borrelia burgdorferi* (Lyme disease) confirmatory test (e.g., Western Blot or immunoblot)
86618	Antibody; *Borrelia burgdorferi* (Lyme disease)
87475	Infectious agent detection by nucleic acid (DNA or RNA*); Borrelia burgdorferi*, direct probe technique
87476	Infectious agent detection by nucleic acid (DNA or RNA); *Borrelia burgdorferi*, amplified probe technique
0041U	Borrelia burgdorferi, antibody detection of 5 recombinant protein groups, by immunoblot, IgM
0042U	Borrelia burgdorferi, antibody detection of 12 recombinant protein groups, by immunoblot, IgG
0316U	Borrelia burgdorferi (Lyme disease), OspA protein evaluation, urine Proprietary test: Lyme Borrelia Nanotrap® Urine Antigen Test Lab/Manufacturer: Galaxy Diagnostics Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
4/26/22	Added CPT code **0316U** (Borrelia burgdorferi (Lyme disease), OspA protein evaluation, urine Proprietary test: Lyme Borrelia Nanotrap® Urine Antigen Test Lab/Manufacturer: Galaxy Diagnostics Inc), which is a not-covered code with Select Health.
10/17/23	Added "Testing" to title of policy to become, "Lyme Disease Testing."

Measurement of Thromboxane Metabolites for ASA Resistance

Policy #: AHS – G2107	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Thromboxane A2 (TXA2) is a metabolite that causes platelet activation in the cyclooxygenase metabolic pathway (Abrams, 2019). Aspirin (ASA) is an acetylated salicylate and is classified as a nonsteroidal anti-inflammatory medication. Aspirin is intended to inhibit cyclooxygenase-1 (COX-1), which then inhibits generation of TXA2, producing the desired antithrombotic effect. Aspirin resistance is the inability of aspirin to decrease platelet production of thromboxane A2 leading to platelet activation and aggregation. (Abramson, 2019).

II. Related Policies

Policy Number	Policy Title
AHS-G2050	Cardiovascular Disease Risk Assessment

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

1) For all indications, the measurement of thromboxane metabolites in urine (e.g., AspirinWorks) to evaluate aspirin resistance **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Aspirin acts primarily by interfering with the biosynthesis of cyclic prostanoids, including thromboxane (Abrams, 2021). It irreversibly inhibits COX-1, resulting in an antithrombotic effect due to a decrease in production of thromboxane. Low doses of aspirin (typically 75 to 81 mg/day) have antiplatelet properties (Abramson, 2021) and are indicated for the primary and secondary prevention of cardiovascular disease. However, aspirin has been noted to occasionally fail to provide any significant benefit in patients with cardiovascular disease. Several possible explanations can account for this phenomenon, such as genetic variability or pharmacological interactions with other drugs, but nonadherence tends to be the most likely cause of nonresponse (Zehnder et al., 2019).

Numerous studies show that aspirin resistance affects 15% to 25% of individuals (Alberts, 2010). A systematic review and meta-analysis on aspirin resistance indicated that patients who are resistant to aspirin are at a greater risk (odds ratio [OR]: 3.85) of clinically important cardiovascular morbidity than patients who are sensitive to aspirin (Krasopoulos et al., 2008). The effect of aspirin administration varies considerably among patients at high risk for cardiovascular events. Gum and colleagues found insufficient inhibition of platelet aggregation by aspirin in 6 to 24% of patients with stable coronary artery disease (Gum et al., 2001) while other estimates range from 5 to 60% (Martin & Talbert, 2005).

Many biochemical tests and several commercially available products have been developed to detect aspirin resistance. Tests used in research laboratories include aggregometry, tests based on activation-dependent changes in platelet surface, and tests based on activation-dependent release from platelets. Point-of-care tests include PFA-100, IMPACT, and VerifyNow, which can detect platelet dysfunction that may be due to aspirin effect (Paniccia et al., 2015). Other tests include Multiplate® analyzer, a multiple electrode aggregometry test (Gillet et al., 2016) and Plateworks assay, a rapid platelet function screening test (Helena_Laboratories, 2021).

It has been proposed that aspirin resistance can also be detected by thromboxane metabolites in urine. Aspirin inhibits platelet activation through the permanent inactivation of the cyclooxygenase (COX) activity of prostaglandin H synthase-1 (COX-1), and consequently inhibits the biosynthesis of thromboxane A2(TXA2), a platelet agonist (Abramson, 2021). The urinary concentrations of the metabolite 11-dehydrothromboxane B2 (11 dhTxB_2) is proposed to indicate the level of TXA2 generation (Smock & Rodgers, 2010).

The AspirinWorks Test Kit is an enzyme-linked immunoassay test that can be used to determine levels of 11 dhTxB2 in human urine (Geske et al., 2008). The AspirinWorks Test Kit was compared to the Accumetrics VerifyNow Aspirin Assay as the predicate device. The manual AspirinWorks Test Kit measures urinary 11 dhTxB2, a metabolite of TxA2, a direct inducer of platelet aggregation while the automated Accumetrics VerifyNow Aspirin Assay is a turbidimetric-based optical detection system,

which measures platelet-induced aggregation in whole blood. Both analyze aspirin's effect through the reduction of TxA2 production or the resulting inhibition of platelet aggregation (FDA, 2015).

A major limitation of this test is that while serum TxB2 comes primarily from platelets, urinary 11dhTxB2 is not a specific measure of platelet thromboxane formation. Urine 11dhTxB2 reflects systemic thromboxane formation, and up to 30% or more can derive from extra-platelet sources, including monocytes, macrophages, atherosclerotic plaque, and other tissues that contain nucleated cells capable of regenerating functional COX-1, or that contain COX-2 (Smock & Rodgers, 2010).

Clinical Validity and Utility

The FDA noted that results from two different clinical studies established a cutoff for aspirin effect at ≤1500 pg 11d hTxB2/mg creatinine. Further analysis revealed that 180/204 (88.2%) of samples from individuals not taking aspirin were above the cut-off value. Analysis of samples from individuals taking various doses of aspirin revealed that 7/163 (4.3%) of 81 mg/day aspirin users indicated a lack of aspirin effect (greater than 1500 pg 1 ldhTxB2/mg creatinine) and 4/38 (10.5%) of the 325 mg/day aspirin users indicated a lack of aspirin effect. In total, 11/201 (5.5%) of all aspirin users tested indicated a lack of aspirin effect (FDA, 2007).

Lordkipanidze et al. (2007) compared the results obtained from six major platelet function tests in the "assessment of the prevalence of aspirin resistance in patients with stable coronary artery disease." 201 patients receiving 80 mg of aspirin were evaluated. Two of the tests used to measure platelet aggregation were VerifyNow and urinary 11-dehydro-thromboxane B(2) concentrations. Prevalence of aspirin resistance for VerifyNow was measured to be 6.7% and 22.9% for urinary 11-dehydro-thromboxane B(2) concentrations. The prevalence of aspirin resistance varied according to the assay used. Results from these tests showed "poor correlation and agreement between themselves." The authors concluded that "platelet function tests are not equally effective in measuring aspirin's anti-platelet effect and correlate poorly amongst themselves and that the clinical usefulness of the different assays to classify correctly patients as aspirin resistant remains undetermined" (Lordkipanidze et al., 2007).

Dretzke et al. (2015) examined "whether or not insufficient platelet function inhibition by aspirin ('aspirin resistance'), as defined using platelet function tests (PFTs), is linked to the occurrence of adverse clinical outcomes, and further, whether or not patients at risk of future adverse clinical events can be identified through PFTs." The authors reviewed 108 studies, with 58 on patients on aspirin monotherapy, and found that some PFTs may have prognostic utility. However, the authors noted that many of the studies found contained significant "methodological and clinical heterogeneity". No cost-effectiveness studies were found.

Wang et al. (2018) evaluated the association between stable urine metabolites of thromboxane (TxA2-M), prostacyclin (PGI2-M), levels of cellular adhesion molecules, chemokines, C-reactive protein, and the incidence of major adverse cardiovascular events (MACE). 120 patients with stable atherosclerotic cardiovascular disease on aspirin therapy were examined. The authors found that urinary TxA2-M levels were "significantly" correlated with circulating P-selectin and E-selectin levels and associated with higher risk of MACE. The authors concluded that "these results provide insight into the contribution of TxA2 biosynthesis to ASCVD progression in humans, and suggest that patients with elevated TxA2-M

levels may be predisposed to advanced platelet and endothelial activation and higher risk of adverse cardiovascular outcomes" (Wang et al., 2018).

Harrison et al. compared 9 platelet function tests to assess responsiveness to three ASA dosing regimens in 24 type 2 diabetes patients randomized to ASA 100 mg/day, 200 mg/day, or 100 mg twice daily for 2 weeks. Of these 9 tests, three were VerifyNow, urinary 11-dehydro-thromboxane B2 (TxB2) and serum TxB2. The investigators evaluated VerifyNow as a "very good" measure, serum TxB2 as a "good" measure, and urinary TxB2 as a "moderate" measure. The authors concluded that "the platelet function tests we assessed were not equally effective in measuring the antiplatelet effect of ASA and correlated poorly amongst themselves, but COX-1-dependent tests performed better than non-COX-1-dependent tests" (Harrison et al., 2018).

Bij de Weg et al. evaluated the changes in aspirin resistance during and after pregnancy. The study focused on "obstetric high risk women with an indication for aspirin usage during pregnancy to prevent placenta mediated pregnancy complications"; in all, 23 pregnant women were included. Four complementary aspirin resistance tests ("PFA-200, VerifyNow®, Chronolog light transmission aggregometry (Chronolog LTA) and serum thromboxane B2 (TxB2) level measurement") were used to measure aspirin resistance in each trimester of pregnancy, as well as 3 months post-partum. The tests identified aspirin resistance at the following: PFA-200: 30.4%, VerifyNow: 17.4%, Chronolog LTA: 26.1%, and serum TxB2, 23.8% respectively. The authors also identified that aspirin resistance tended to be more frequency during pregnancy compared to after pregnancy. However, the authors also acknowledged that there was "weak" correlation between tests and recommended more research on aspirin resistance as well as obstetric outcome (Bij de Weg et al., 2020).

Ebrahimi et al. performed a meta-analysis focusing on laboratory-defined aspirin resistance rate in cardiovascular disease patients. 65 studies encompassing 10729 patients were evaluated. The overall prevalence of laboratory-defined aspirin resistance was measured to be 24.7%, with women at slightly higher risk for resistance compared to men (odds ratio = 1.16). The authors also found that higher prevalence of resistance tended to be found in Asia, whereas American studies found the lowest rates of resistance. The authors recommended that providers pay attention to potential aspirin resistance in their patients (Ebrahimi et al., 2020).

Singh et al. (2021) investigated the use of miR-19b-1-5p as a biomarker for aspirin resistance in acute coronary syndrome (ACS) patients as an alternative to in-vitro platelet function tests, which have potential limitations in detection. MiR-19b-1-5p expression was measured in 945 patients with ACS and platelet function was determined by multiplate aggregometry testing. Low miR-19b-1-5p expression was found to be related to aspirin resistance, which agreed with the sustained platelet aggregation in the presence of aspirin. "Therefore, miR-19b-1-5p could be a suitable marker for aspirin resistance and might predict recurrence of future major adverse cardio-cerebrovascular events in patients with ACS" (Singh et al., 2021).

Piao et al. (2021) compared the performance of the Anysis-200 analyzer and VerifyNow assays to assess platelet inhibition in cardiac disease patients. In relation to VerifyNow, the sensitivity (96.3%) and specificity (90.3%) of Anysis-200 was comparable. The aspirin resistance rate in patients was 20.9% using VerifyNow and 16.5% using Anysis-200. The Cohen's kappa coefficient between the two devices was 0.81, indicating an almost perfect agreement between the two devices. Overall, the Anysis-200 assay "would

be used as a point-of-care test to assess aspirin non-responsiveness and abnormal platelet reactivity" (Piao et al., 2021).

V. Guidelines and Recommendations

Pan-European, multidisciplinary Task Force for Advanced Bleeding Care in Trauma

This Task Force includes representatives from six different societies: The European Society for Trauma and Emergency Surgery (ESTES), the European Society of Anaesthesiology (ESA), the European Shock Society (ESS), the European Society for Emergency Medicine (EuSEM), the Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis (NATA) and the European Society of Intensive Care Medicine (ESICM). Although this guideline focuses on trauma settings, there are some comments on point-of-care (POC) platelet function tests, such as VerifyNow. The Task Force remarks that:

- "The role of POC platelet function devices in guiding haemostatic therapy is not established".
- "Currently, there is no agreement on the optimal assay for platelet function (see R11) and controversy exists as to whether bleeding in the setting of APA [aspirin] use warrants platelet transfusion", although the Task Force acknowledges that "that reliable measures of platelet function would be useful to guide reversal therapies in the setting of the bleeding trauma patient".
- The Task Force also states that due to the "lack of congruency" demonstrated by studies focusing on these platelet function assays, there is a need for future studies to investigate the potential benefit of these platelet function monitoring assays. The Panel remarks that "their [platelet function assays]' role in identifying trauma-induced platelet dysfunction and in guiding haemostatic therapy remains unclear and their use can only be recommended as an adjunct to standard laboratory monitoring".
- Overall, the following recommendation of "We suggest the use of POC platelet function devices as an adjunct to standard laboratory and/or POC coagulation monitoring in patients with suspected platelet dysfunction" was given a grade of "2C", which was defined as "Very weak recommendation; other alternatives may be equally reasonable" (Spahn et al., 2019).

International Society on Thrombosis and Haemostasis

The Working Group on Aspirin Resistance (Michelson et al., 2005) published a position paper which concluded that other than in research trials it is not appropriate to test for aspirin resistance or change therapy based on such tests. There are no published studies which address the clinical effectiveness or data linking aspirin dependent laboratory test to clinical outcomes in patients (Michelson et al., 2005).

Study Group on Biomarkers in Cardiology of the Acute Cardiovascular Care Association and the Working Group on Thrombosis of the European Society of Cardiology

This study group was convened to assess the utility of platelet function testing in acute cardiac care for predicting adverse events and guiding antiplatelet therapy. The panel lists recommended assays for assessment of platelet activity during $P2Y_{12}$ inhibitors, which are "the VASP-P® assay, the VerifyNow® device and the Multiplate® analyser". Although VerifyNow is the precursor to AspirinWorks, AspirinWorks itself was not mentioned as a recommended assay (Aradi et al., 2015).

American College of Chest Physicians Evidence-Based Clinical Practice Guidelines

The ACCP states "the clinical significance of [platelet function] assay findings is uncertain, and the assay results have not been shown to predict clinical outcomes" (Douketis et al., 2012).

The American Society of Anesthesiologists (ASA)

The ASA released guidelines on platelet function testing in patients on antiplatelet therapy before cardiac surgery. The ASA advises that platelet function testing may be considered to guide decisions on timing of cardiac surgery in patients who have recently received P2Y12 receptor inhibitors or who have ongoing dual antiplatelet therapy. The ASA highlights the advantages and disadvantages of various platelet function assays and notes that the underlying principles between the assays differ therefore, a poor correlation has been reported between the assays. Currently, there is not enough evidence from surgical patients to prefer one test over the other. Among the various methods, the ASA recommends Thromboelastography (TEG5000, TEG6s) with platelet mapping assay or the VerifyNow P2Y12 assay as both are more appropriate in coronary artery surgery patients since it can be performed at bedside (Mahla et al., 2020).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

VerifyNow-Aspirin Assay, which received 510(k) marketing clearance from the FDA in October 2004, is a qualitative assay to aid in the detection of platelet dysfunction due to aspirin ingestion in citrated whole blood for the point of care or laboratory setting (FDA, 2004).

AspirinWorks received 510(k) marketing clearance from the FDA in May 2007 and is intended to aid in the qualitative detection of aspirin in apparently healthy individuals post ingestion.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82570	Creatinine; other source
84431	Thromboxane metabolite(s), including thromboxane if performed, urine

VIII. Evidence-based Scientific References

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Metabolite Markers of Thiopurines Testing

Policy #: AHS – G2115	**Prior Policy Name & Number (as applicable):** AHS-G2115 Pharmacogenomic and Metabolite Markers for Thiopurines
Implementation Date: 9/15/21	**Date of Last Revision:** 11/12/21, 2/16/23 (See Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Thiopurines are a class of purine antimetabolite immunomodulators with diverse clinical applications in treatment of autoimmune disorders, transplant rejection, and acute lymphoblastic leukemia (Belmont, 2019; MacDermott, 2019). Their therapeutic efficacy, bone marrow toxicity, and liver toxicity have been reported to be related to levels of their downstream metabolites. Due to their complex metabolism, patient response varies considerably between individuals, both in achieving therapeutic drug levels as well as in developing adverse reactions (Bradford & Shih, 2011).

Please note that this policy discusses the monitoring of thiopurine metabolite levels in individuals. For guidance on pharmacogenetic testing prior to therapy, please refer to AHS-M2021 Pharmacogenetic Testing.

II. Related Policies

Policy Number	Policy Title
AHS-M2021	Pharmacogenetic Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1) One-time phenotypic analysis of the enzyme TPMT **MEETS COVERAGE CRITERIA** in the following situations:
   a) In patients prior to initiating treatment with azathioprine (AZA), mercaptopurine (6-MP) or thioguanine (6-TG).
   b) In patients on thiopurine therapy with abnormal complete blood count (CBC) results that do not respond to dose reduction.

2) In individuals with inflammatory bowel disease, monitoring of thiopurine metabolite levels **MEETS COVERAGE CRITERIA** for the following indications:
   a) To measure blood levels in individuals suspected of having toxic responses to AZA and/or 6-MP (e.g., hepatotoxicity or myelotoxicity).
   b) To measure drug levels in individuals who have not responded to therapy (e.g., persistent fever, further weight loss, and bloody diarrhea).

3) In individuals with acute lymphoblastic leukemia, monitoring of thiopurine metabolite levels **MEETS COVERAGE CRITERIA** in the following situations:
   a) For patients showing signs of a lack of myelosuppression while on therapy.
   b) For patients who do not tolerate thiopurines.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4) Phenotypic analysis of the enzyme TPMT **DOES NOT MEET COVERAGE CRITERIA** in all other situations.

5) Analysis of the metabolite markers of azathioprine and 6-mercaptopurine, including 6-methyl-mercaptopurine ribonucleotides (6-MMRP) and 6-thioguanine nucleotides (6-TGN), **DOES NOT MEET COVERAGE CRITERIA** in all other situations.

IV. Scientific Background

The thiopurine drugs 6-mercaptopurine (6-MP), azathioprine (AZA), and thioguanine remain a mainstay of immunomodulator therapy (Belmont, 2019; Tantisira, 2019). However, several metabolites of these drugs (particularly 6-thioguanine [6-TG] and 6-methylmercaptopurine [6-MMP]) have been associated with harmful side effects, such as lowered therapeutic efficacy, hepatotoxicity, bone marrow toxicity, and more. The management of these toxic metabolites is further complicated by the many polymorphisms (and therefore efficacy in metabolism) of the genes responsible for metabolizing these drugs. Due to these toxic side effects, there has been significant investigation on monitoring of these metabolites to identify the optimal dose of a thiopurine for an individual patient. This process is called therapeutic drug monitoring (TDM) Rubin, 2020).

Two enzymes are responsible for catalyzing these reactions: thiopurine methyltransferase (TPMT) and hypoxanthine phosphoribosyl transferase. TPMT enzyme activity is a major factor determining AZA and 6-MP metabolism, and therefore 6-TG and 6-MMP levels. The majority of the population has wild type *TPMT* and normal enzyme activity, while 11% are heterozygous and have corresponding low TPMT enzyme activity, and 0.3% (1 in 300) have negligible activity (Lennard et al., 1993; Lennard et al., 1989). Intermediate and normal metabolizers can have up to a threefold difference in initial target doses of AZA and 6-MP to achieve therapeutic 6-TG concentrations (Gardiner et al., 2008). Measurement of TPMT enzyme activity before instituting AZA or 6-MP may help prevent toxicity by identifying individuals with low or absent TPMT enzyme activity as well as identify those with higher than average TPMT activity who may remain refractory to conventional dosages (Rubin, 2020).

Dosing strategies involving such testing may be cost-effective (Cuffari et al., 2004; Dubinsky et al., 2005; Winter et al., 2004). However, prediction of toxicity is not consistently reliable, as other enzymes are also likely to play a role in determining toxicity, such as glutathione-S-transferase (Stocco et al., 2007), and drug interactions must be taken into account (Dewit et al., 2002; Gilissen et al., 2005; Szumlanski & Weinshilboum, 1995). Thus, even though TPMT testing is recommended (Relling et al., 2019), a complete blood count (CBC), and also liver function tests, must still be obtained (Belmont, 2019).

Another enzyme that may contribute to thiopurine metabolism is nucleoside diphosphate-linked moiety X motif 15, NUDIX 15 (NUDT15). Variants in this enzyme's genotype and subsequent phenotype may lead to drastically reduced tolerance of 6-MP (Tantisira, 2019). Moriyama et al. proposed that NUDT15 variants cause thiopurines' mechanism of action to fail by preventing the thiopurine metabolites' incorporation into DNA. This causes these metabolites to remain active and therefore toxic (Moriyama et al., 2016). The frequency of these NUDT15 variants varies across populations, with the "poor metabolizer" phenotype reaching as high as 1 in 50 in East Asian populations. Despite the data indicating NUDT15's role in thiopurine toxicity, guidelines for its assessment have not reached a consensus, and expert opinions and practices are mixed (Tantisira, 2019).

Therapeutic drug monitoring (TDM) is the measurement of serum, plasma, or urinary concentrations of a given drug. This can be measured in a variety of ways, including high performance liquid chromatography (HPLC) or mass spectrometry approaches such as GC-MS (Eadie, 1998). TDM is proposed to allow a clinician to identify the "optimal" dose of a drug (such as a thiopurine) for a patient,

thereby maximizing therapeutic efficacy and minimizing harmful side effects. Non-TDM approaches typically involve starting at low doses, then adjusting if the patient is tolerating the drug well or poorly, whereas TDM takes a more proactive approach in managing dose (Rubin, 2020). Several studies have attempted to identify standardized ranges of "optimal" metabolite concentrations. For example, the optimal concentration of the 6-TGN metabolite was found to be between 230 and 400 picomoles per 8 x 10^8 erythrocytes by Dubinsky et al. In that same study, bone marrow toxicity was found to correlate with levels above 400 picomoles per 8 x 10^8 erythrocytes (Dubinsky et al., 2000). Although there are potential limitations to TDM for thiopurines (such as intra-individual variability, lack of correlation with toxicity for 6-MMP, and so on), TDM used in conjunction with TPMT and NUDT15 assessment may allow clinicians to increase the therapeutic efficacy of thiopurines (Rubin, 2020; Tantisira, 2019).

Clinical Utility and Validity

Haines et al. (2011) performed a retrospective study of the utility of measuring thiopurine metabolites in "inadequately controlled" inflammatory bowel disease (IBD). 63 patients with IBD were included, and these patients were treated with AZA or 6-mercaptopurine. On weight-based criteria, 50% patients were underdosed. However, metabolite study suggested that "7 (11%) patients were noncompliant, 18 (29%) were being underdosed, 33 (52%) were refractory to treatment with either appropriate (41%) or elevated (11%) metabolite concentrations, and 6 (10%) had a raised 6-methyl mercaptopurine: 6-thioguanine nucleotide ratio consistent with aberrant thiopurine metabolism". The clinical outcome of 87% of patients improved when the treatment was shifted to a metabolite-based algorithm, whereas 3 of 17 patients improved when the discordant action was taken. The authors concluded that "Thiopurine metabolite testing is a potentially powerful tool for optimizing thiopurine usage in IBD" (Haines et al., 2011).

Lee et al. evaluated 165 patients undergoing thiopurine treatment for Crohn's Disease. Thiopurine metabolite levels were measured, and both *TPMT* and *NUDT15* were genotyped. The authors found 95 patients responded to treatment whereas 45 did not. The median 6-TGN (the primary metabolite of 6-thioguanine) was significantly higher in responders than nonresponders. At a 6-TGN level of 230 pmol/8 x 10^8 blood cells, the odds ratio was 4.63 for responders to nonresponders. *NUDT15* variants were also found to be associated with "severe, early, leukopenia" with an average reduction of 88.2% from baseline white blood cell count at 4 weeks. The authors concluded that their findings "support the role of therapeutic drug monitoring in thiopurine maintenance treatment to optimize thiopurine therapy, especially, for non-responding CD patients" (Lee et al., 2017).

Spencer et al. (2019) compared "standard" and "optimized" thiopurine dosing regimens in 216 pediatric IBD patients. The "optimal" level was decided at "6-TGN >235 pmol/8 × 10^8 RBC", and the metabolite levels were correlated between the primary outcome of "steroid-free clinical remission (SFR)". Both groups were found to have similar initial and 6-month metabolite levels. SFR was achieved in 74% of the 180 patients on thiopurines at 6 months. The authors concluded that "steroid-free clinical remission and 6-TGN levels at 6 months were no different between a standardized, fixed dosing strategy and a metabolite-driven, optimized dosing strategy" (Spencer et al., 2019).

Meijer et al. evaluated the effects of thiopurine metabolites on clinical signs and if patient characteristics affected metabolite generation. 940 "laboratory findings" from 424 patients were examined. 6-TGN (a metabolite of azathioprine [AZA] and mercaptopurine) was found to negatively correlate with RBC count, WBC count, and neutrophil count. However, in patients using 6-thioguanine,

those 6-TGN concentrations correlated positively with WBC count. An inverse correlation was observed between age and 6-TGN concentrations in AZA or 6-thioguanine patients, as well as between body mass index and 6-TGN in AZA or mercaptopurine patients. The authors concluded that "thiopurine derivative therapy influenced bone marrow production and the size of red blood cells. Age and body mass index were important pharmacokinetic factors in the generation of 6-TGN" (Meijer et al., 2017).

Estevinho et al. performed a meta-analysis to "assess the clinical value of 6-thioguanine nucleotide thresholds; and ii] to compare mean 6-thioguanine nucleotide concentrations between patients in clinical remission vs. those with active disease." A total of 22 records were used in cut-off comparisons and 12 were used in the 6-thioguanine nucleotide mean differences analysis. The authors calculated the global odds ratio for remission in patients with 6-thioguanine nucleotides above predefined thresholds to be 3.95. The authors also found an odds ratio for remission of 2.25 for the 235 pmol/8 x 10^8 RBC threshold, and an odds ratio of 4.71 for the 250 pmol /8 x 10^8 RBC threshold. Finally, the authors found a "pooled difference" of 63.37 pmol/8 x 10^8 RBC between patients in clinical remission and those not in remission. Overall, the authors concluded that the study reinforced the link between and 6-thioguanine nucleotide levels and clinical remission in inflammatory bowel diseases (Estevinho et al., 2017).

Toksvang et al. performed a meta-analysis focusing on "incidence of hepatotoxicity in patients [with childhood acute lymphoblastic leukaemia, ALL or inflammatory bowel disease, IBD] treated with 6TG [6-thioguanine]". 42 reports were included, further broken down into "four randomised controlled trials (RCTs) including 3,993 patients, 20 observational studies including 796 patients, and 18 case reports including 60 patients". The authors measured hepatotoxicity by "sinusoidal obstruction syndrome", which occurred in 9-25% of ALL patients in two of the four RCTs at a dosage of 40–60 mg/m^2/day. The authors also noted that at a dosage of 23 mg/m^2/day, nodular regenerative hyperplasia (NRH) occurred in 14% of IBD patients. At a dosage of 12 mg/m^2/day, NRH occurred in 6% of IBD patients, which was noted to be similar to background incidence. The authors therefore concluded that doses at or under 12 mg/m^2/day can "probably be considered safe" (Toksvang et al., 2019).

Zhu et al. evaluated the "predictive sensitivity based on 6TGN [6-thioguanine nucleotide] by subgrouping patients according to their *NUDT15* R139C genotypes". The authors included 411 patients with Crohn's Disease. Two subgroups of *NUDT15* genotypes were created, "CC" (n = 342) and "CT" (n = 65), with the final four patients harboring a TT genotype. Thiopurine-induced leukopenia (TIL) was the primary clinical endpoint measured. The authors found that of the 342 patients with a CC genotype, only 35 developed TIL (10.2%), but of the 65 CT patients, 33 developed TIL (50.2%). All four of the TT patients developed TIL. The authors also found that in both CC and CT genotypes, the median 6TGH level was higher in patients with TIL than patients without TIL (for CC, 474.8 pmol/8 × 10^8 RBC vs 306.0 pmol/8 × 10^8 RBC, for CT 291.7 / 8 × 10^8 RBC vs 217.6/8 × 10^8 RBC). From this data, the authors calculated the "cut-off" (a threshold to identify an optimal number of cases) of the CT genotype to be 319.2 pmol/8 × 10^8 RBC and the cut-off for CC to be 411.5 pmol/8 × 10^8 RBC). Overall, the authors concluded that "The predictive sensitivity of TIL based on 6TGN is dramatically increased after subgrouping according to *NUDT15* R139C genotypes. Applying 6TGN cut-off levels to adjust thiopurine therapies based on *NUDT15* is strongly recommended" (Zhu et al., 2019).

V. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

In version 2 of the 2021 guidelines for Pediatric Acute Lymphoblastic Leukemia, the NCCN recommends that "for patients homozygous for normal function TPMT and NUDT15, who do not appear to tolerate thiopurines, consider measuring erythrocyte thiopurine metabolites. Genetic testing may fail to identify rare or previously undiscovered no function alleles". The NCCN also writes that "genetic testing for no function alleles of TPMT and NUDT15 should be considered prior to the initiation of thiopurine therapy" (NCCN, 2019, 2021b).

In version 2 of the 2021 guidelines for Acute Lymphoblastic Leukemia, the NCCN notes that "quantification of 6-MP metabolites can be very useful in determining whether lack of myelosuppression is due to non-compliance or hypermetabolism"

The 2021 guidelines also state, "for patients receiving 6-MP, consider testing for TPMT gene polymorphisms, particularly in patients who develop severe neutropenia after starting 6-MP. Testing for both TPMT and NUDT15 variant status should be considered, especially for patients of East Asian origin" (NCCN, 2021a).

Toronto Ulcerative Colitis Consensus Group/American College of Gastroenterology (ACG)

Bressler et al. (2015) published clinical practice guidelines for the medical management of non-hospitalized ulcerative colitis on behalf of the Toronto Ulcerative Colitis Consensus Group, which reaffirmed recommendations from the American College of Gastroenterology, Practice Parameters Committee (Kornbluth & Sachar, 2010) for thiopurine therapy (Bressler et al., 2015). The authors stated that "…a TPMT assay is necessary before initiation of treatment to identify patients at risk for severe dose-dependent myelosuppression…therefore, thiopurine metabolite levels may be helpful to guide therapy. Note that TPMT testing does not replace the need for mandatory monitoring of complete blood cell count" (Bressler et al., 2015).

American College of Gastroenterology (ACG)

The ACG published a guideline for the "Management of Crohn's Disease in Adults". Their relevant recommendations include:

"Thiopurine methyltransferase (TPMT) testing should be considered before initial use of azathioprine or 6-mercaptopurine to treat patients with Crohn's disease (strong recommendation, low level of evidence)" (Lichtenstein et al., 2018).

The ACG also published a guideline for ulcerative colitis (UC) in adults. Their relevant recommendations include:

"The patient with nonresponse or loss of response to therapy should be assessed with therapeutic drug monitoring to identify the reason for lack of response and whether to optimize the existing therapy or to select an alternate therapy."

"There is insufficient evidence supporting a benefit for proactive therapeutic drug monitoring in all unselected patients with UC in remission" (Rubin et al., 2019).

North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) Committee

In 2013, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) Committee on Inflammatory Bowel Disease published consensus recommendations on the role of TPMT and thiopurine metabolite testing in pediatric IBD (Benkov et al., 2013) The recommendations included the following:

- "TPMT testing is recommended before initiation of TPs to identify individuals who are homozygote recessive or have extremely low TPMT activity, with the latter having more reliability than the former. (HIGH)."
- "Individuals who are homozygous recessive or have extremely low TPMT activity should avoid use of TPs because of concerns for significant leukopenia. (HIGH)"
- "TPMT testing does not predict all cases of leukopenia and has no value to predict hypersensitivity adverse effects such as pancreatitis. Any potential value to reduce the risk of malignancy has not been studied. All individuals on TPs should have routine monitoring with CBC and WBC count differential to evaluate for leukopenia regardless of TPMT testing results. (HIGH)"
- "Metabolite testing can be used to determine adherence to TP therapy. (HIGH)"
- "Metabolite testing can be used to guide dose increases or modifications in patients with active disease. Consideration would include either increasing the dose, changing therapy or for those with elevated transaminases or an elevated 6-MMP, using adjunctive allopurinol to help raise 6-TG metabolites and suppress formation of 6-MMP. (MODERATE)"
- "Routine and repetitive metabolite testing has little or no role in patients who are doing well and taking an acceptable dose of a TP. (MODERATE)"

American Gastroenterological Association (AGA)

In 2017, the AGA published guidelines (J. D. Feuerstein et al., 2017) on Therapeutic Drug Monitoring in Inflammatory Bowel Disease which recommend:

- "In adult patients treated with thiopurines with active IBD or adverse effects thought to be due to thiopurine toxicity, the AGA suggests reactive thiopurine metabolite monitoring to guide treatment changes."
- "In adult patients with quiescent IBD treated with thiopurines, the AGA suggests against routine thiopurine metabolite monitoring."

The AGA published an Institute Technical Review on the Role of Therapeutic Drug Monitoring in the Management of Inflammatory Bowel Diseases in the same year. In it, they note that IBD patients treated with thiopurines may benefit from reactive TDM to guide treatment changes (Vande Casteele et al., 2017).

In the 2020 AGA guidelines for "Management of Moderate to Severe Ulcerative Colitis", the AGA remarks that "therapeutic drug monitoring to guide the use of biologic therapy has been addressed in

separate AGA guidelines". The "separate AGA guidelines" refer to the 2017 edition above (Feuerstein et al., 2020).

Clinical Pharmacogenetics Implementation Consortium (CPIC)

In their guideline for "Thiopurine Dosing Based on TPMT and NUDT15 Genotypes", CPIC notes that "mercaptopurine and azathioprine are generally used for nonmalignant immunologic disorders, mercaptopurine for lymphoid malignancies, and thioguanine for myeloid leukemias". However, CPIC also writes that "variants in NUDT15 have been identified that strongly influence thiopurine tolerance in patients with acute lymphoblastic leukemia (ALL) and those with inflammatory bowel diseases" (Relling et al., 2019).

- "For TPMT and NUDT15 indeterminate phenotypes, (i.e. combination of uncertain and/or unknown function alleles):
  - TPMT indeterminate: Consider evaluating TPMT erythrocyte activity to assess TPMT phenotype.
  - NUDT15 indeterminate: If thiopurines are required and NUDT15 status is unknown, monitor closely for toxicity" (CPIC, 2020).

British Society of Gastroenterology (BSG)

The BSG published "consensus guidelines" on management of inflammatory bowel disease in adults. They recommend checking TPMT status in "all patients considered for thiopurine therapy". They also recommend testing the NUDT15 genotype if "available".

The BSG also writes that thiopurine metabolites should be checked if a patient experiences myelotoxicity as a side effect. Similarly, if a patient demonstrates "newly abnormal LFTs [liver function tests]", thiopurine metabolites should be checked. Also, BSG states that "all IBD patients considered for thiopurine therapy should have assessment of thiopurine methyltransferase (TPMT) status." (Lamb et al., 2019).

Overall, the BSG writes that thiopurine metabolites can be used to "optimize drug dosing" and "suggest that metabolite monitoring may be used for those with inadequate response to therapy or toxicity, but should not be a substitute for routine monitoring blood tests" (Lamb et al., 2019).

Canadian Association of Gastroenterology

The Canadian Association of Gastroenterology published a guideline on "the Medical Management of Pediatric Luminal Crohn's Disease". The guidelines "suggested that testing for TPMT by genotype or enzymatic activity be done prior to initiating thiopurine therapy to guide dosing" (Mack et al., 2019).

National Institute for Health and Care Excellence (NICE)

NICE, released guidelines on Crohn's Disease in 2019. In it, they recommend to "Monitor the effects of azathioprine, mercaptopurine, and methotrexate as advised in the British national formulary (BNF) or British national formulary for children (BNFC)" (NICE, 2019).

European Crohn's and Colitis Organization and European Society of Paediatric Gastroenterology, Hepatology and Nutrition

These joint guidelines note that "measuring…6-TG and 6-MMP levels after 2–3 months, may aid in optimizing thiopurine dosing". Measuring thiopurine metabolites is recommended in the following scenarios:

- In patients with incomplete response on stable thiopurine dosage
- In patients who present with leucopenia or elevated transaminases
- After acute severe colitis (ASC) responsive to intravenous corticosteroids (IVCS) (Turner et al., 2018)
- When poor compliance is suspected (Turner et al., 2018).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82657	Enzyme activity in blood cells, cultured cells, or tissue, not elsewhere specified; nonradioactive substrate, each specimen

VIII. Evidence-based Scientific References

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Onychomycosis Testing

Policy #: AHS – M2172	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Onychomycosis, also known as tinea unguium (Wollina, Nenoff, Haroske, & Haenssle, 2016), is a fungal infection of the nail typically caused by pathogenic fungal dermatophytes, such as Trichophyton rubrum, Trichophyton mentagrophytes, and Epidermophyton floccossum; onychomycosis may also be caused by yeasts, including Candida parapsilosis and Candida guilliermondii, or non-dermatophyte molds, including Neoscytalidium dimidiatum, Onychocola canadensis, the Aspergillus species, Scopulariopsis species, Alternaria species, Acremonium species, and Fusarium species (Ameen, Lear, Madan, Mohd Mustapa, & Richardson, 2014; Bongomin, Batac, Richardson, & Denning, 2018; Wollina et al., 2016).

II. Related Policies

Policy Number	Policy Title
AHS-G2149	Pathogen Panel Testing
AHS-M2097	Identification of Microorganisms Using Nucleic Acid Probes

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clipping(s) **MEETS COVERAGE CRITERIA** for individuals with onychomycosis.

2. The use of nucleic acid tests* (See Note 1), including but not limited to PCR, PCR-RFLP, and next-generation sequencing (NGS), to screen for, diagnose, or confirm onychomycosis **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3. The use of attenuated total-reflectance Fourier transform infrared (ATR-FTIR) spectroscopy to screen for, diagnose, or confirm onychomycosis **DOES NOT MEET COVERAGE CRITERIA**.
4. Testing for the presence of fungal-derived sterols, including but not limited to ergosterol, **DOES NOT MEET COVERAGE CRITERIA**.

Note 1: Nucleic acid testing of following microorganisms: *Candida* species, *Aspergillus* species, *Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccossum, Neoscytalidium dimidiatum, Onychocola canadensis, Scopulariopsis* species, *Alternaria* species, *Acremonium* species, and *Fusarium* species (Ameen et al., 2014; Bongomin et al., 2018; Wollina et al., 2016).

IV. Scientific Background

Onychomycosis is a fungal infection of the nail that causes approximately 50% of nail disease cases (Gupta, Versteeg, & Shear, 2017) and is considered the most common nail disorder based on clinical statistics (Lipner & Scher, 2019). Onychomycosis infections can be obtained through several sources, including hotel carpets, bathtubs, saunas, pool decks, and public showers, and may be generated by dermatophytes, yeast, or mold. Data show that toenails are impacted 25 times more often than fingernails (Bongomin et al., 2018), and the first and fifth toe nail are more likely to be infected owing to the fact that footwear more frequently damages these nails (Ameen et al., 2014).

Dermatophytes are pathogenic fungi that can infect the skin, hair, and/or nails (Koo et al., 2019), and they are estimated to cause 90% of onychomycosis toenail cases and 50% of fingernail cases (Bodman & Krishnamurthy, 2019). These fungi attach to a surface such as an epithelial cell, extract nutrients, and grow as hyphae or filaments forming molds; this process allows the dermatophyte to seed several conditions, including onychomycosis (tinea unguium), athlete's foot (tinea pedis), and scalp ringworm (tinea capitis) (Achterman & White, 2013). Wollina et al. (2016) suggest that an estimated 68% of onychomycosis cases are due to dermatophytes, 29% of cases due to yeasts, and 3% due to molds; further, mixed flora were identified in 5-15% of cases. Several types of dermatophytes may produce an onychomycosis infection, including *Trichophyton rubrum*, *Trichophyton mentagrophytes,* and *Epidermophyton floccossum* (Bodman & Krishnamurthy, 2019). In the United Kingdom, 85-90% of nail infections are due to dermatophytes (Ameen et al., 2014), while non-dermatophyte molds are estimated to cause between 2-25% of all onychomycosis cases (Bongomin, Batac, Richardson, & Denning, 2018). Non-dermatophyte mold onychomycosis causative agents include the *Aspergillus* species; incidence rates with this species vary between 1-35% of all cases and almost 71% in the elderly population (Bongomin et al., 2018).

A mature nail is comprised of the nail bed, nail plate, nail matrix, and nail fold (Wollina et al., 2016). Onychomycosis-causing pathogens live on the keratin of dead corneocytes and primarily infect the nail bed; after the nail bed thickens or becomes hyperkeratotic, the nail matrix is damaged (Bodman & Krishnamurthy, 2019). The nail plate may also be invaded during the infection, eventually becoming detached or warped, allowing the affliction to intensify (Bodman & Krishnamurthy, 2019). If a toenail

case is not treated, the fungi, mold, or yeast could spread to the foot, causing tinea pedis in appropriate conditions; infections may also spread to the hands or groin area (Ameen et al., 2014). If skin is externally disrupted, allowing bacteria entry into the body, the infection may also cause foot ulcers, cellulitis, osteomyelitis, and gangrene in diabetic patients (Ameen et al., 2014). While an official diagnosis requires lab results, typical visual cues for an onychomycosis infection include a jagged edge of the infected area of the nail "with spikes directed to the proximal fold, white-yellow longitudinal striae in the onycholytic nail plate, and colored parallel bands" (Abdallah, Said, Mahmoud, & Omar, 2019). Subungual short spikes are also indicative of onychomycosis (Bodman & Krishnamurthy, 2019).

Several types of onychomycosis have been identified and include distolateral subungual (DLSOM), superficial white, proximal subungual, endonyx, and total dystrophic (TDOM) onychomycosis (Abdallah et al., 2019). Superficial white onychomycosis is rare, develops only in toenails, and occurs when the pathogens invade the nail through the nail plate; in proximal subungual onychomycosis, the infection occurs through the cuticle and typically develops in patients with a suppressed immune system (Wollina et al., 2016). Endonyx onychomycosis, which is caused by *T. soudanense,* occurs when the nail plate thickens; finally, the most advanced stage of onychomycosis is TDOM which may take up to 10 or 15 years to develop and can mature from any of the four main onychomycosis types mentioned above (Wollina et al., 2016).

The global prevalence of onychomycosis is estimated at 5.5% of the total population (Gupta, Versteeg, & Shear, 2017). Ameen et al. (2014) estimate the onychomycosis prevalence in the United Kingdom at 3% of the adult population, while Wollina et al. (2016) estimate the prevalence in both the United States and Europe at 4.3% of the total population. Further, studies with a hospital-based population report an incidence at 8.9% (Wollina et al., 2016). Both lifestyle and general climate can impact infection rates.

As onychomycosis causes approximately 50% of nail disease cases, an estimated 15% of nail disorders can be contributed to metabolic conditions or inflammatory disorders, and 5% due to malignancies or pigment ailments (Wollina et al., 2016). Non-infectious nail diseases may include lichen ruber, yellow nail syndrome, psoriasis unguium, and tumors (Wollina et al., 2016). Onychomycosis may be stimulated by other nail disorders such as psoriasis (Ghannoum et al., 2018). When compared to nail psoriasis, onychomycosis infections tend to have more layers of parakeratosis, a greater amount of neutrophils and serous lakes, and a more blurred and/or irregular nail transition zone than psoriasis-based infections (Trevisan, Werner, & Pinheiro, 2019).

Several ailments or conditions increase the risk of an onychomycosis infection, including diabetes, obesity, old age, immunosuppression, smoking, human immunodeficiency virus (HIV) (Gupta et al., 2017), and cancer; further, patients who receive dialysis or who have previously received a transplant also experience a greater risk of developing an onychomycosis infection (Wollina et al., 2016). Diabetics are almost three times more likely to develop onychomycosis than non-diabetics; current data suggests that an estimated 34% of all diabetics have been diagnosed with the ailment (Ameen et al., 2014). Patients with HIV typically experience a more severe infection with all fingernails and toes infected due to a compromised immune system (Ameen et al., 2014). Onychomycosis is rare in pediatric populations, except in children with Down syndrome or immunodeficiencies (Solis-Arias & Garcia-Romero, 2017). Both men and older adults are more likely to develop onychomycosis compared to females or young adults (Ameen et al., 2014). These statistics could be contributed to the fact that older adults are more likely to exhibit reduced peripheral circulation, larger and potentially abnormal nail surfaces, difficulty grooming and maintaining efficient hygiene levels, and may have a greater chance of exposure to pathogenic fungi (Ameen et al., 2014). Athletes also experience onychomycosis infections at a greater

incidence, with data suggesting that athletes are 2.5 times more likely to develop an infection than the general population, with infections seven times more prevalent in toenails than fingernails (Daggett, Brodell, Daniel, & Jackson, 2019). This is likely due to the warm and moist environment in the shoe and sock, close quarters with other athletes, and/or trauma to the foot during sporting activities.

An onychomycosis diagnosis should be given based on both clinical results and mycological lab results (Wollina et al., 2016). Several types of tests have been developed to diagnose onychomycosis. The current diagnostic gold standard includes direct microscopy with potassium hydroxide (KOH) and fungal culture, as these methods can identify the pathogenic species and fungal viability; additional tests include polymerase chain reaction (PCR) testing, fluorescent staining and periodic acid-Schiff (PAS) staining (Gupta et al., 2017; Rios-Yuil, 2017). It has been reported that KOH testing is only 60% sensitive and cannot identify the species, but it can differentiate between dermatophytes and saprophytes based on a positive result; "Currently, the most sensitive test (95%) is a pathologist interpreted nail clip biopsy that has been stained with periodic acid-Schiff (PAS) plus Grocott methenamine silver (Bodman & Krishnamurthy, 2019)." Mycologic culture may be used for suspected onychomycosis cases with negative KOH results if spores, hyphae, or other fungal structures were seen via microscopy; histologic evaluation of a nail clipping using PAS stain may assist in an onychomycosis diagnosis with more sensitive results than those given by mycologic culture (Arndt, LeBoit, & Wintroub, 2016). An *Aspergillus* species causative agent may be suspected with a negative culture result but a positive KOH test (Bongomin et al., 2018). Fungal cultures must be interpreted by a mycologist and, while they are specific, they are only about 60% sensitive and take several weeks to grow (Bodman & Krishnamurthy, 2019). When utilized together, fungal culture and PCR can determine the source of the infection; the addition of PCR can improve species detection by 20% and will assist in differentiating between onychomycosis and nail dystrophy. PCR, when used with fungal culture, allows for a "much faster, highly sensitive, and very specific diagnosis" (Wollina et al., 2016). Multiplex qPCR assays have shown to be reliable for onychomycosis diagnostics with a shorter response time than traditional culture methods (Koo et al., 2019).

Many commercial tests are available.

For example, a multi-component test developed by Ipsum Diagnostics uses PCR to quickly identify the disease-causing agent in an onychomycosis infection alongside additional histology testing methods to provide same day results and evidence-based treatment options for both bacterial and fungal species (Ipsum_Diagnostics, 2020).

SSI Diagnostica has developed a commercial Dermatophyte Real Time PCR Kit which allows for the diagnostic detection of dermatophytes in nail samples, particularly *T. rubrum* (SSI, 2020).

LabCorp has developed a fungus (mycology) culture test which analyzes a nail sample for an onychomycosis infection and delivers results in 24-42 days (LabCorp, 2020).

Ability Diagnostics offers a similar nucleic acid test, which detects 11 different fungal species purported to cause fungal infections (Ability_Diagnostics, 2020).

NovaDX offers a nucleic acid test akin to the tests listed above, although the specific pathogens tested for are not listed (NovaDX, 2020).

MicroGenDX offers a next-generation sequencing test to identify both bacterial and fungal species for nail infections. The test also provides a corresponding antibiotic list, based on antibiotic resistance genes detected. The test also prioritizes 16 items for 24-hour rapid results, which are as follows: "Methicillin

resistance, Vancomycin resistance, Beta-lactam [resistance], Carbapenem [resistance], Macrolide [resistance], Aminoglycoside [resistance], Tetracycline [resistance], *Enterococcus faecalis Streptococcus agalactiae* (group B), *Streptococcus pyogenes* (group A), *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Trichophyton rubrum*" (MicroGenDX, 2020).

Finally, Vikor Scientific has developed the Nail-ID™ test which uses advanced molecular PCR technology to deliver rapid results "through a value-based technology platform, ABXAssist™, which incorporates regional sensitivity and susceptibility patterns, medication costs, antibiotic spectrum of activity, and FDA guidance (Vikor, 2020)." The Nail-ID™ is able to deliver results in 24 hours after the sample is received, can detect polymicrobial infections simultaneously, and may identify as many as 49 antibiotic resistance genes to assist with treatment regimens (Vikor, 2020).

Current onychomycosis treatments encompass antifungal medications (i.e. tavaborole and efinaconazole) and laser therapy; other treatments in the pipeline include iontophoresis and photodynamic therapy (Gupta et al., 2017). Dermatophyte infections may be treated with fluconazole, terbinafin, or itraconazole, while *Candida spp.* infections respond best to fluconazole (Wollina et al., 2016). Oral antifungal treatments are effective, but typically cause several unwanted side effects; on the other hand, topical antifungal treatments are less effective due to difficulties penetrating the nail, but cause minimal side effects (Leung et al., 2019). If the nail matrix is involved, which can typically be identified by yellow streaks tarnishing the nail, both a systemic and topical antimycotic drug are recommended (Wollina et al., 2016). Treatments may occur over a period of months or years before an improvement is noticed; further, a toenail onychomycosis infection is reportedly more difficult to treat than a fingernail infection, and a recurrence rate is estimated between 5-50% (Bodman & Krishnamurthy, 2019). An article by Gupta et al. (2019) report that a relapse is likely to occur within the first 2.5 years after the infection has been cured; moreover, they state that to maximize cure rates, biofilms should be disrupted, drugs with more than one route of delivery should be utilized, and non-traditional treatments should be used in a timely manner if initial treatments are not efficient. Preventive strategies include retaining clean footwear, keeping toenails short and using topical antifungal agents.

Other fungal infections, such as dermatophytoma, may occur with onychomycosis infections, making these infections harder to treat; dermatophytoma can typically be identified "as a dense concentration of fungal hyphae within or under the nail plate and is generally white or yellow/brown in color, and linear (streaks) or round (patches) in shape (Aly, Winter, Hall, & Vlahovic, 2018)." A classification system has been developed to categorize the severity of an onychomycosis infection, termed the Onychomycosis Severity Index (OSI) (Carney et al., 2011). This score is determined by "multiplying the score for the area of involvement (range, 0-5) by the score for the proximity of disease to the matrix (range, 1-5). Ten points are added for the presence of a longitudinal streak or a patch (dermatophytoma) or for greater than 2 mm of subungual hyperkeratosis. Mild onychomycosis corresponds to a score of 1 through 5; moderate, 6 through 15; and severe, 16 through 35 (Carney et al., 2011)."

Clinical Validity

Fungal fluorescent staining and internal transcribed spacer (ITS) ribosomal DNA (rDNA) PCR sequencing methods were compared to traditional direct microscopy with KOH detection methods for onychomycosis diagnostics; data from a total of 204 patients was used (Bao et al., 2018). Fungal

fluorescent staining was found to have a sensitivity of 97% and a specificity of 89%, while ITS rDNA PCR had a sensitivity of 78% and a specificity of 90%; the researchers concluded that the "Use of fluorescence enhanced the sensitivity of direct examination by 12% compared with KOH. PCR-based sequencing increased the sensitivity by 6% compared with culturing (Bao et al., 2018)."

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is a PCR technique that can be used to diagnose onychomycosis developed by Lubis, Muis, and Nasution (2018); this method was compared against the fungal culture gold standard. Samples were collected from 35 patients; this PCR-RFLP method was found to have a specificity of 28.57% and a sensitivity of 85.71% (Lubis et al., 2018). While the sensitivity is high, a low specificity may suggest that this technique be used alongside the gold standard for onychomycosis testing to further improve sensitivity instead of replacing the traditional diagnostic method altogether.

Joyce, Gupta, Koenig, Wolcott, and Carviel (2019) measured the effectiveness of quantitative PCR and next-generation sequencing instead of traditional, but expensive, KOH and culture techniques in diagnosing 8,816 "clinically suspicious" toenail samples; approximately 50% of the toenail samples were found to contain fungi and bacteria. The authors stated that these "Molecular methods were successful in efficiently quantifying microbial and mycologic presence in the nail. Contributions from dermatophytes were lower than expected, whereas the opposite was true for nondermatophyte molds (Joyce et al., 2019)."

Gustafson, Bakotic, Bennett, Page, and McCarthy (2019) used a real-time PCR assay on 425 clinical samples of suspected onychomycosis; results were compared to traditional KOH microscopy results. "Of 425 clinical samples suspected of onychomycosis analyzed by fungal culture and PCR, 219 samples were positive for both (52% agreement). Of the 206 discordant samples, 95% were resolved in favor of PCR by DNA sequencing (Gustafson et al., 2019)." These researchers also analyzed a larger data set of 2,452 samples. It was identified that histopathology has a positivity rate of 85%, PCR had a positivity rate of 73% and culture had a positivity rate of 54%; "PCR outperformed culture compared to histopathology for sensitivity (80% versus 49%), specificity (92% versus 79%), positive predictive value (94% versus 77%), and negative predictive value (76% versus 52%) (Gustafson et al., 2019)."

De Bruyne et al. (2019) used attenuated total-reflectance Fourier transform infrared (ATR-FTIR) spectroscopy as an alternative method to diagnose onychomycosis; spectral differences were used for dermatophytes (1692-1606 and 1044-1004 cm^{-1}) as well as for nondermatophytes and yeasts (973-937 cm^{-1}). An accuracy rating of 96.9% was given when identifying between uninfected nails, and nails infected with either dermatophytes, yeasts, or nondermatophytes; further, when discriminating between dermatophytes, yeasts, and nondermatophytes, classification rates were given of 91.0%, 98.6% and 97.7% respectively (De Bruyne et al., 2019).

Liquid chromatography-tandem mass spectrometry has been used by Ho, Li, and Yang (2019) to identify ergosterol, a sterol that most fungi cannot survive without, as a new diagnostic tool for fungal infected nails. Samples from 20 participants were collected and analyzed, which is a relatively small sample size. However, the researchers determined that this mass spectrometry diagnostic method "seemed to be better at detecting combinations of nail conditions" than current techniques, but further studies need to be completed to determine the sensitivity and specificity of this method (Ho et al., 2019).

Mourad, Ismail, Hawwam, Msseha, and Hassan (2019) compared Chicago sky blue staining and Calcofluor white staining to traditional KOH wet mount and culture techniques; samples from 50 patients with dermatophytosis of the hair or nail were used. Both Chicago sky blue staining and

Calcofluor white staining of the hair and nail were found to be more specific and sensitive for the diagnosis of fungal infections when compared to traditional diagnostic methods because the KOH wet mount technique is reportedly a "simple, rapid, and inexpensive test but lacks color contrast and gave more false positive (artifacts) and false-negative results as compared to these new stain methods (Mourad et al., 2019)."

Clinical Utility

The frequency of onychomycosis infections was measured in patients with psoriasis compared to controls by Romaszkiewicz et al. (2018); data from a total of 2527 patients was used, with 2325 patients presenting with nail abnormalities and onychomycosis suspicion with no previous history of psoriasis, 102 psoriatic patients with onychomycosis suspicion, and 100 controls. The researchers used direct microscopy and culture to identify fungal infections, and found that "The prevalence of onychomycosis did not differ significantly between psoriatic patients and non-psoriatic patients with nail alterations (Romaszkiewicz et al., 2018)." However, it was identified that the characteristics of the fungi isolated from the patients "differed significantly between psoriatic and non-psoriatic patients", which is important to note regarding treatment regimens (Romaszkiewicz et al., 2018). Another study, completed by Gallo, Cinelli, Fabbrocini, and Vastarella (2019), also measured onychomycosis prevalence between psoriatic and non-psoriatic patients; similar results were found. This study analyzed data from a total of 9281 patients and found similar infection rates between psoriatic and non-psoriatic groups; however, once again, the "spectrum of fungal species isolated was different," with patients in the non-psoriatic group more likely to be infected with yeasts than patients in the psoriatic group (Gallo et al., 2019).

A meta-analysis was completed by Velasquez-Agudelo and Cardona-Arias (2017) to determine the utility, validity and performance of culture, nail clippings with PAS staining, and KOH testing for onychomycosis diagnostic purposes; this meta-analysis search utilized "5 databases and 21 search strategies." Results showed that "The diagnostic tests evaluated in this meta-analysis independently showed acceptable validity, performance, and efficiency, with nail clipping with PAS staining outperforming the other two tests (Velasquez-Agudelo & Cardona-Arias, 2017)." Another study by Gupta, Versteeg, and Shear (2018) measured several types of onychomycosis confirmatory testing methods such as KOH, culture, and PAS. It was determined that PAS was once again "the most sensitive confirmatory test and KOH the least expensive"; incorrect diagnoses made without confirmatory tests led to the unnecessary spending of several hundred Canadian dollars, suggesting that confirmatory lab diagnostics are preferred before treatment (Gupta et al., 2018).

Martinez-Herrera, Arroyo-Camarena, Tejada-Garcia, Porras-Lopez, and Arenas (2015) measured the number of onychomycosis cases due to opportunistic molds; this retrospective study analyzed data from 4220 onychomycosis cases and found that only 32 cases (0.76%) were caused by opportunistic molds. This study also found that the age group most affected was between 41 and 65 years old and that females were affected slightly more than males at 65.6% (Martinez-Herrera et al., 2015). Further, the authors also reported that "The most frequent isolated etiological agents were: *Aspergillus sp.* and *Scopulariopsis brevicaulis* (Martinez-Herrera et al., 2015)."

Haghani, Shams-Ghahfarokhi, Dalimi Asl, Shokohi, and Hedayati (2019) examined the species distribution of "causative agents" of onychomycosis. A total of 257 patients contributed samples, and the agents in these samples were identified through PCR. Onychomycosis was identified in 180 cases, and "51.1% of these cases were caused by non-dermatophyte moulds (NDMs), 35% by yeast and 10.6%

by dermatophytes." The authors also found that novel triazoles and imidazoles such as "efinaconazole, luliconazole and lanoconazole" showed "potent" activity compared to other antifungal agents. The authors concluded that "that obtained data will be useful to improve the knowledge of researchers, clinicians and dermatologists about onychomycosis distribution, species diversity and adoption of appropriate treatment." (Haghani et al., 2019)

V. Guidelines and Recommendations

Centers of Disease Control and Prevention (CDC, 2020)

The CDC remarks that an onychomycosis infection may be diagnosed through visual inspection, questioning the patient on their symptoms, or a fungal culture. No mention was made of molecular-based testing or PCR testing. The CDC also notes that the term "onychomycosis" is the technical term for a "fungal nail infection" (CDC, 2020).

American Academy of Pediatrics (AAP, 2018a, 2018b)

Within the AAP's Red Book, recommendations include the following concerning diagnostic testing for onychomycosis: "Fungal infection of the nail (tinea unguium or onychomycosis) can be verified by direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clippings fixed in formalin (AAP, 2018b)."

The AAP also notes that confirmatory diagnostic tests are similar to those for tinea corporis. According to the AAP Red Book, fungal culture to diagnose tinea corporis can be used, but that "polymerase chain reaction and periodic acid-Schiff stain evaluation of specimens are available but are expensive and generally are not necessary (AAP, 2018a)."

British Association of Dermatologists (BAD) (Ameen et al., 2014)

The BAD have published guidelines for the management of onychomycosis stating that "The clinical characteristics of dystrophic nails must alert the clinician to the possibility of onychomycosis. Laboratory confirmation of a clinical diagnosis of tinea unguium should be obtained before starting treatment. This is important for several reasons: to eliminate nonfungal dermatological conditions from the diagnosis; to detect mixed infections; and to diagnose patients with less responsive forms of onychomycosis, such as toenail infections due to *T. rubrum*. Good nail specimens are difficult to obtain but are crucial for maximizing laboratory diagnosis. Material should be taken from any discoloured, dystrophic or brittle parts of the nail (Ameen et al., 2014)."

Further, the BAD also stated that "Traditionally, laboratory detection and identification of dermatophytes consists of culture and microscopy, which yields results within approximately 2–6 weeks. Calcofluor white is exceedingly useful for direct microscopic examination of nail specimens, as the fungal elements are seen much more easily than with potassium hydroxide, thereby increasing sensitivity (Ameen et al., 2014)."

More recent molecular genetic tools were also highlighted as a newer diagnostic technique for the detection of dermatophytes. Regarding PCR testing, the BAD has stated that "Real-time polymerase chain reaction (PCR) assays have been developed, which simultaneously detect and identify the most prevalent dermatophytes directly in nail, skin and hair samples and have a turnaround time of < 2 days. It appears that real-time PCR significantly increased the detection rate of dermatophytes compared with culture. However, PCR may detect nonpathogenic or dead fungus, which could limit its use in identifying the true pathogen. Restriction fragment length polymorphism analysis, which identifies fungal ribosomal

DNA, is very helpful for defining whether the disease is caused by repeat infection or another fungal strain when there is a lack of response to treatment. However, this technique has not been implemented into routine clinical practice (Ameen et al., 2014)."

Finally, the BAD also stated that "histopathological analysis using periodic acid–Schiff staining is more sensitive than direct microscopy or culture. However, this technique is not currently available in the majority of dermatology clinics or mycology laboratories. Other diagnostic techniques under investigation include flow cytometry and confocal and scanning electron microscopy (Ameen et al., 2014)."

Canadian Paediatric Society (CPS) [(Bortolussi & Martin, 2007) reaffirmed 2019]

The CPS notes that treatment effectiveness will differ depending on the type of fungal or mold infection, and therefore highlights the importance of sending nail clippings for culture to "allow differentiation between dermatophyte and non-dermatophytic fungal nail infections." The CPS also remarks that "Terbinafine has excellent action against dermatophytes, but is less effective for *Candida* onychomycosis, and these cases are best treated with azoles" (Bortolussi & Martin, 2007).

The American Academy of Family Physicians (AAFP) (Ely, Rosenfeld, & Seabury Stone, 2014; Westerberg & Voyack, 2013)

The AAFP published guidelines in 2013 regarding current trends in the diagnosis and treatment of onychomycosis. These guidelines suggested C evidence ratings for the following statements:

- "Periodic acid–Schiff staining should be ordered to confirm infection in patients with suspected onychomycosis
- When preparing a nail specimen to test for onychomycosis, the nail should be cleaned with 70% isopropyl alcohol, then samples of the subungual debris and eight to 10 nail clippings should be obtained (Westerberg & Voyack, 2013)."

The AAFP also stated that an "Accurate diagnosis is crucial for successful treatment and requires identification of physical changes and positive laboratory analysis (Westerberg & Voyack, 2013)." Further, a diagnosis flowchart was given and states that if a nail is discolored or gives reason to suspect onychomycosis, nail clippings should be obtained and looked at under a microscope; if the microscopic viewing suggests a positive onychomycosis diagnosis, treatment should begin to identify the organism (treatment includes culture and/or histologic evaluations with periodic acid-Schiff staining) (Westerberg & Voyack, 2013).

In 2014, Ely et al. (2014) gave a C evidence rating to both "Tinea corporis, tinea cruris, and tinea pedis can often be diagnosed based on appearance, but a potassium hydroxide preparation or culture should be performed when the appearance is atypical" and "The diagnosis of onychomycosis should generally be confirmed with a test such as potassium hydroxide preparation, culture, or periodic acid–Schiff stain before initiating treatment."

VI. State and Federal Regulations (as applicable)

A search of the FDA database on 1/25/2021 using the terms "onychomycosis" or "tinea unguium" yielded 0 results. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82542	Column chromatography, includes mass spectrometry, if performed (e.g., HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
87101	Culture, fungi (mold or yeast) isolation, with presumptive identification of isolates; skin, hair, or nail
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87153	Culture, typing; identification by nucleic acid sequencing method, each isolate (e.g., sequencing of the 16S rRNA gene)
87205	Smear, primary source with interpretation; Gram or Giemsa stain for bacteria, fungi, or cell types
87206	Smear, primary source with interpretation; fluorescent and/or acid fast stain for bacteria, fungi, parasites, viruses or cell types
87220	Tissue examination by KOH slide of samples from skin, hair, or nails for fungi or ectoparasite ova or mites (e.g., scabies)
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique

87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique
88312	Special stain including interpretation and report; Group I for microorganisms (e.g., acid fast, methenamine silver)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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IX. Revision History

Revision Date	Summary of Changes

Oral Cancer Screening and Testing

Policy #: AHS – G2113	**Prior Policy Name & Number (as applicable):** Oral Screening Lesion Identification Systems and Genetic Screening (AHS-G2113)
Implementation Date: 9/15/21	**Date of Last Revision:** 8/22/22, 10/24/23 (see Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Oral cancer is defined as cancer occurring in the oral cavity between the vermilion border of the lips and the junction of the hard and soft palates or the posterior one third of the tongue. Squamous cell carcinoma is the most common type of oral cancer (Gross et al., 2023).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For individuals with oropharyngeal squamous cell carcinoma, testing for high-risk HPV with either mRNA expression testing or immunohistochemistry for p16 expression **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

2. To screen, detect, or diagnose oral cancer, the following testing **DOES NOT MEET COVERAGE CRITERIA:**

a) Salivary biomarker testing (e.g., peptides/proteins, nucleic acids, metabolites).

b) Genotyping of HPV (e.g., OraRisk® HPV).

c) Gene expression profiling.

d) Panels that incorporate genetic risk factors with nongenetic biomarkers (e.g., mRNA CancerDetect™).

III. Scientific Background

The American Cancer Society (ACS) estimates the 2019 incidence of oral cancer to be 53,000 cases with approximately 10,860 deaths (Siegel et al., 2019). The American Cancer Society estimates that in the United States in 2023, approximately 54,540 people will be diagnosed with oral cavity and oropharyngeal cancers and approximately 11,580 people will die from these cancers (ACS, 2023). Oral squamous cell carcinoma (OSCC) is the most common form of oral cavity cancer, which constitutes 94.08% of all epithelial tumors and 80.05% of all oral cancers (Dhanuthai et al., 2018; Scully & Porter, 2000). Many cases are preceded by a potentially malignant disorder (PMD), which is a heterogeneous group of conditions including erythroplakia, non-homogeneous leukoplakia, erosive lichen planus, oral submucous fibrosis and actinic keratosis (Warnakulasuriya et al., 2007). The early detection and excision of PMD can prevent malignant transformation (Paul Brocklehurst, 2017; van der Waal, 2009; Warnakulasuriya et al., 2007).

Human papillomavirus (HPV) is a common sexually transmitted infection that may lead to the development of warts or cancer in various parts of the body including the back of the throat, tonsils and base of the tongue. This type of cancer is known as oropharyngeal cancer. HPV is also a major contributor to the development of head and neck squamous cell carcinoma (HNSCC), which can develop in the mouth, nose and throat (Borsetto et al., 2018). According to the CDC (2022), there is no test to determine an individual's HPV status, and "there is no approved HPV test to find HPV in the mouth or throat."

Oral Screening Lesion Identification Systems and Genetic Screening

Diagnosing and treating dermatologic lesions of the mouth and gums is challenging for most clinicians because of the wide variety of disease processes that can present with similar appearing lesions and the fact that most clinicians receive inadequate training in mouth diseases (Lodi, 2023). Several index tests have been proposed as adjuncts to a conventional oral examination (COE) to improve diagnostic test accuracy (Fedele, 2009; Lingen et al., 2008; Patton et al., 2008; Rethman et al., 2010; Seoane Leston & Diz Dios, 2010). These tests include vital staining, brush cytology, light-based detection, and blood or saliva analysis. The COE is the standard visual and tactile exam of the mucosa under normal light. Although it is quick and minimally invasive, abnormalities may not be malignant or even visible (Macey et al., 2015). These screening tests are not only used for diagnostic purposes but can also be utilized as a tool to measure any changes that may be signs of future disease development (Speight et al., 2017).

Finally, blood or saliva can be tested for biomarkers for cancer. The tests are non-invasive but have low standardization and are not widely used in clinical practice (Macey et al., 2015). Nonetheless, saliva has been identified as an ideal diagnostic medium for the early detection of HNSCC activity because it is close to the tumor site and is an easy sample to obtain (Lim et al., 2016). Macey et al. (2015) concluded that none of the adjunctive biomarker tests can be recommended as a replacement for the currently used standard of COE followed by a scalpel biopsy and histological assessment. However, the NCCN has stated that that "Expression of p16 as detected by IHC [immunohistochemistry] is a widely available surrogate biomarker that has a very good agreement with HPV status as determined by the gold standard of HPV E6/E7 mRNA expression" (NCCN, 2023). The protein known as p16 slows cell division, therefore acting as a tumor suppressor. Researchers have identified $p16^{INK4a}$, RASSF1A, TIMP3, and PCQAP/MED15 as tumor suppressor genes that exhibited "excellent diagnostic accuracy in the early detection of OC [oral cancer] at 91.7% sensitivity and 92.3% specificity and of OPC [oropharyngeal cancer] at 99.8% sensitivity and 92.1% specificity from healthy controls" (Liyanage et al., 2019). A review by Kaur, Jacobs, Huang, Salvo, and Politis (2018) that researched salivary biomarkers for oral cancer and pre-cancer screening have identified a plethora of salivary biomarkers which showed an improvement in oral cancer diagnoses including mRNAs, salivary transcriptomes (IL-8, IL-IB, DUSP1, HA3, OAZ1, S100P, and SAT were highly specific (91%) and sensitive (91%) for oral cancer detection), and salivary biomarkers (M2BP, profilin, CD59, MRP14, and catalase had a sensitivity of 83% and a specificity of 90% for oral cancer detection) (Kaur et al., 2018)."

The OraRisk® HPV by OralDNA Labs is a salivary diagnostic test that analyzes the molecular genotypes of HPV. The test can identify a total of 51 types of oral HPV including high-risk, low-risk and unknown-risk genotypes. High Risk Genotypes: 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 64, 66, 67, 68, 69, 70, 73, 82. Low Risk Genotypes: 2a, 6, 11, 32, 40, 42, 43, 44, 54, 55, 57, 61, 62, 71, 72, 74, 77, 81, 83, 84, 89. Unknown Risk Genotypes: 41, 49, 60, 75, 76, 80, 85 (OralDNA, 2023).

Clinical Utility and Validity

Nagi et al. (2016) conducted a systematic review to evaluate the effectiveness of adjunctive devices that utilize the principles of chemiluminescence and tissue autofluorescence in the detection of oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD). Twenty primary studies published satisfied the criteria for selection. Ten used chemiluminescence and 10 used tissue autofluorescence. ViziLite was used for evaluation of chemiluminescence, and it was evaluated at a sensitivity of 0.771 to 1.00 and specificity of 0.00 to 0.278. Tissue autofluorescence was evaluated with VELscope. This technique was evaluated at a sensitivity of 0.22-1.00 and specificity of 0.16 to 1.00. The authors concluded that more clinical trials in the future should be conducted to establish optical imaging as an efficacious adjunct tool in early diagnosis of OSCC and OPMD (Nagi et al., 2016).

Shaw et al. (2022) conducted a systematic review to compare the existing evidence on diagnostic accuracy of salivary biomarkers with their estimation method in detecting early oral squamous cell carcinoma. Salivary biomarkers provide promising complementary alternative diagnostic adjunct for its simple non-invasive collection and technique and to screen large population. "18 studies were included for qualitative synthesis, and out of that 13 for meta-analysis. Sensitivity and specificity were calculated with AUC. For mRNA it was 91% and 90% with 0.96 AUC, miRNA had 91% and 91% with 0.95 AUC for PCR. IL-1B had 46%

and 60% with 0.61 AUC, S100p had 45% and 90% with 0.57 AUC for ELISA. IL-8 had 54% and 74% for ELISA and 89% and 90% for PCR with 0.79 AUC and DUSP1 had 32% and 87% for ELISA and 76% and 83% for PCR with 0.83 AUC respectively. Early detection of OSCC was best achieved by screening for salivary mRNA and miRNA estimated by PCR" (Shaw et al., 2022).

Lingen et al. (2017); Lingen et al. (2008) performed a meta-analysis of the screening adjuncts for oral cancer. The authors evaluated cytologic adjuncts as well as vital staining, tissue reflectance, autofluorescence, and salivary biomarkers. The vital staining cohort included 15 studies with 1453 lesions and was evaluated at a 0.87 sensitivity and 0.71 specificity. The tissue reflectance cohort (5 studies, 390 lesions) was assessed at a 0.72 sensitivity and 0.31 specificity. The autofluorescence segment (7 studies, 616 lesions) was computed at a 0.90 sensitivity and a 0.72 specificity. The authors stated, "most biomarkers showed a wide range of diagnostic test accuracy results, with sensitivity ranging from 0.5 to 0.9 and specificity ranging from 0.63 to 0.9." Finally, cytology (15 studies, 2148 lesions) was assessed at a 0.92 sensitivity and 0.94 specificity. The authors concluded that cytology appeared to be most accurate adjunct (Lingen et al., 2017).

Another systematic review was completed that focused on the use of oral brush cytology for the early detection of oral cancer and OPMDs (Alsarraf, Kujan, & Farah, 2018). Thirty-six of the 343 abstracts and articles identified met the inclusion criteria, with publication dates ranging from 1994 to 2017. These articles led to the inclusion of 4302 total samples from OPMDs, oral squamous cell carcinoma, and healthy controls. The results were somewhat troubling. "Findings from this study indicate that meaningful evidence-based recommendations for the implementation of a minimally invasive technique to be utilized as an adjunctive tool for screening and early detection of oral cancer and OPMDs are complicated from the reported studies in the literature (Alsarraf et al., 2018)."

Kaur et al. (2018) completed a review which focused on salivary biomarkers for oral cancer and pre-cancer screening. A total of 270 articles published between 1995 and 2017 were identified for this review. The authors note that biomarkers may be arranged into four categories: normal health (IL-8, IL-1beta, etc.), general health (glycolytic enzyme lactate dehydrogenase, etc.), specific (S100P mRNA for cancer), and non-specific salivary (8-OHdG and MDA biomarkers of oral cancer and pre-cancer) (Kaur et al., 2018). Results from this study led to the conclusion that "Biomarkers such as methylation markers, IL-8, actin, myosin, and miRNAs are very speculative and remain without sufficient scientific evidence when it comes to oral cancer and pre-cancer detection using body fluids. Salivary peptides such as protein 14, Mac-2 binding protein, profilin 1, CD59, defensin-1, catalase proteins, etc. with sensitivity approximating 90% and specificity 80% for oral cancer diagnosis have been described"; "Furthermore, five salivary metabolites such as valine, lactic acid, and phenylalanine in combination yielded satisfactory accuracy (0.89), sensitivity (94.6%), and specificity (84.4%) in distinguishing oral cancer from controls or oral pre-cancer, respectively (Kaur et al., 2018)." Based on the results in this large group of studies, the researchers state that the "Combination approach of salivary biomarkers could be used as [a] screening tool to improve early detection and diagnostic precision of oral pre-cancer and cancer (Kaur et al., 2018)." The findings of this extensive review highlight that it is important for researchers to mitigate the current challenges involved with the use of salivary biomarkers for oral cancer and pre-cancer screening as this technique has the potential to improve early detection and diagnostic methods.

Using "targeted proteomics, identified initially by relative quantification of salivary proteins on LC-MS [light chromatography-mass spectrometry]," Jain et al. (2021) identified a potential salivary biomarker panel having been motivated by the high prevalence, incidence, and mortality of oral cancer/oral squamous cell carcinoma among Indians. In a case-control cohort study, "Out of the twelve proteins validated, two proteins AHSG and KRT6C were significantly upregulated and four proteins, AZGP1, KLK1, BPIFB2 and LACRT were found to be significantly downregulated," but when accounting for tobacco consumption habits, "AHSG and AZGP1 were dysregulated in cases compared to controls irrespective of their tobacco consumption habits. While KRT6C, KLK1 and BPIFB2 were significantly dysregulated only in the cases having tobacco consumption habits." AZGP1 is important in insulin sensitivity and the cell cycle; KLK1 is a serine protease involved in "remodelling of the extracellular matrix, cellular proliferation and differentiation, angiogenesis, and apoptosis;" BPIFB2 is a lipid transfer/lipopolysaccharide binding protein that is not well understood in cancer; KRT6C is a type II keratin subtype and is expressed in "filiform papillae of the tongue, stratified epithelial lining of the oesophagus, and oral mucosa and in glandular epithelia;" and AHSG is involved in "multiorgan expression during embryogenesis," but is mostly in the liver and some osteoblasts in adults. In their risk prediction model, AZGP1, AHSG, and KRT6C had sensitivities of 82.4%, 78%, and 73.5%, respectively for all stages of OSCC, and 87.9%, 87.5%, and 73.5%, respectively for late stage OSCC. This could prove future benefits of utilizing salivary proteins in staging oral cancer and OSCC in certain patient populations.

Lim et al. (2016) competed a study to determine the diagnostic ability of four HNSCC biomarkers (RASSF1α, $p16^{INK4a}$, TIMP3, PCQAP/MED15) isolated from saliva. The DNA methylation status of these biomarkers was measured via methylation-specific PCR (MSP). Data from a total of 88 HNSCC patients and 122 healthy controls was analyzed. The authors found that a "Salivary DNA tumour-suppressor methylation gene panel has the potential to detect early-stage tumours in HPV-negative HNSCC patients. HPV infection was found to deregulate the methylation levels in HPV-positive HNSCC patients"; biomarker analysis of HPV-negative HNSCC patients compared to healthy controls generated a sensitivity of 71% and specificity of 80%, while biomarker analysis of HPV-positive HNSCC patients compared to healthy controls generated a sensitivity of 80% and a specificity of 74% (Lim et al., 2016).

In their overview of non-invasive diagnostic devices in oral oncology, Mascitti et al. (2018) discussed and reviewed the Vizilite® chemiluminescence-based detected device for PMD and OSCC (Zila Pharmaceuticals), VELscope® non-magnifying device for visualization of oral mucosa autofluorescence (LED Medical Diagnostics), Identafi® device for multispectral screening of PMD (StarDEntal-DentalEZ), Microlux/DL™ chemiluminescence-based device (AdDent Inc.), GOCCLES® device for autofluorescence abnormalities in the oral cavity (Pierrel S.p.A), Orascoptic DK™ chemiluminscence-based device (Orascoptic), and other autofluorescence-based devices like those from Sapphire® PLUS LD (DenMat Holdings), DentLight DOE™ Oral Exam System (DentLight), and ORalID™ 2.0 (Forward Science Technologies). Ultimately, they concluded that there would be "great potential for screening and monitoring lesions. Unfortunately, to date several factors hinder an extensive use of these devices: (1) data do not demonstrate clear superiority of these methods compared to COE; (2) there remains the need for well-designed multicentre prospective studies; (3) these devices exhibit a not-negligible interobserver variability limiting their use to clinicians with significant experience in oral pathology." However, in terms of their benefits, "the current evidence suggests that these devices: (1) seem to be

useful in assessing lesion margins that must be biopsied and, therefore, may be useful in surgical management; (2) can be used to investigate biological aspects of oral carcinogenesis, leading to more accurate methods for interpreting data from LBDS [light-based detection systems]; (3) can be enhanced with new approaches used to analyse optical imaging data, with the aim to quantify the results obtained; (4) lowering the costs of these devices could indirectly lead to greater attention for oral lesions among both patients and general dental practitioners, allowing in turn to promote a culture of oral cancer prevention; (5) finally, the possibility of implementing LBDS through the use of tissue-marking dyes can in principle allows to develop strategies for the use of nanoparticles. Indeed, nanoparticles can provide molecular targeted imaging, with higher image contrast and resolution" (Mascitti et al., 2018).

Ribeiro et al. (2021) conducted a study aiming to identify prognostic biomarkers for OSCC using a whole genome technology and evaluate their clinical utility. With using array comparative genomic hybridization technology from 62 patients with OSCC, they found that the "chromosomes most commonly altered were 3p, 3q, 5q, 6p, 7q, 8p, 8q, 11q, 15q, 17q, and 18q", with a greater frequency of alterations found on 3p, 3q, 8p, 8q, and 11q. To differentiate between patients with and without metastases or relapses after primary treatment, the researchers identified a genomic signature of genes including *OCLN, CLDN16, SCRIB, IKBKB, PAK2, PIK3CB,* and *YWHAZ*; this rendered an overall accuracy of 79%. An amplification of the *PIK3CB* gene also predicted metastases and relapses in addition to reducing median survival by more than 5 years. This demonstrated the potential use of genes in developing precision medicine and treating patients with OSCC (Ribeiro et al., 2021).

IV. Guidelines and Recommendations

US Preventive Services Task Force (USPSTF)

In 2013, the USPSTF published final recommendations for screening of oral cancer. The recommendation stated that "the current evidence is insufficient to assess the balance of benefits and harms of screening for oral cancer in asymptomatic adults." The USPSTF also noted that "although there is interest in screening for oral HPV infection, medical and dental organizations do not recommend it" (Moyer, 2014).

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines on head and neck cancers does not mention the use of adjunctive screening aids based on autofluorescence or tissue reflectance as a management tool (NCCN, 2023). Regarding biomarker testing, the NCCN states that "A few HPV testing options are available for use in the clinical setting. Expression of p16 as detected by IHC [immunohistochemistry] is a widely available surrogate biomarker that has a very good agreement with HPV status as determined by HPV E6/E7 mRNA expression." They also state, "P16 expression is highly correlated with HPV status and prognosis and is widely available". HPV testing by p16 IHC is a required portion of the workup of the cancer of the oropharynx algorithm, such that "Expression of p16 as detected by IHC is a widely available surrogate biomarker that has very good agreement with HPV status as determined by HPV E6/E7 mRNA expression" (NCCN, 2023).

College of American Pathologists (CAP)

The CAP published guidelines on human papillomavirus testing in head and neck carcinomas. These guidelines state that "For oropharyngeal tissue specimens (ie, noncytology), pathologists should perform HR-HPV [high-risk HPV] testing by surrogate marker p16 IHC" (Lewis et al., 2018).

American Society of Clinical Oncology (ASCO)

An expert panel from the ASCO has "determined that the recommendations from the HPV Testing in Head and Neck Carcinomas guideline, published in 2018, are clear, thorough, and based upon the most relevant scientific evidence. ASCO endorsed the [CAP] guideline and added minor qualifying statements" (Fakhry et al., 2018).

The ASCO states that "It is recommended that HPV tumor status should be determined for newly diagnosed oropharyngeal squamous cell carcinomas. HPV tumor status testing may be performed by surrogate marker p16 immunohistochemistry either on the primary tumor or from cervical nodal metastases only if an oropharyngeal primary tumor is present" (Fakhry et al., 2018).

Regarding diagnosis and management of squamous cell carcinoma of unknown primary (SCCUP) in the head and neck, the ASCO states with a moderate strength recommendation, "High-risk (HR) human papillomavirus (HPV) testing should be done routinely on level II and III SCCUP nodes. Epstein-Barr virus (EBV) testing should be considered on HPV-negative metastases… HR-HPV testing may be done nonroutinely for SCC metastases at other nodal levels when the clinical suspicion is high" (Maghami et al., 2020).

European Head and Neck Society (EHNS)-European Society for Medical Oncology (ESMO)-European Society for Radiotherapy and Oncology (ESTRO)

In 2020, the EHNS, ESMO, and ESTRO released joint clinical practice guidelines for squamous cell carcinoma of the oral cavity, larynx, oropharynx, and hypopharynx. For HPV testing, they recommended that "for SCCHN [squamous cell carcinoma of the head and neck] of unknown primary, p16 and EBER [Epstein-Barr-encoded RNA] are recommended. If p16 staining is positive, another specific HPV test should be carried out to confirm the HPV status [III, A]." p16 measured by immunohistochemistry is validated in use as a surrogate marker for HPV-induced oropharyngeal cancer and prognostic factor for oropharyngeal cancer [I, A] (Machiels et al., 2020)

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82397	Chemiluminescent assay
87623	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), low-risk types (e.g., 6, 11, 42, 43, 44)
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (e.g., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)
87625	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
8/22/22	Removed coverage criteria #2a (MOP testing).
10/24/23	The following changes were implemented, changed title of policy to to "Oral Cancer Screening and Testing" (was previously titled "Oral Screening Lesion Identification Systems and Genetic Screening); former coverage criteria #2 and #3 were combined into a single requirement, which now reads: "2) To screen, detect, or diagnose oral cancer, the following testing **DOES NOT MEET COVERAGE CRITERIA:** a) Salivary biomarker testing (e.g., peptides/proteins, nucleic acids, metabolites). b) Genotyping of HPV (e.g., OraRisk® HPV). c) Gene expression profiling. d) Panels that incorporate genetic risk factors with nongenetic biomarkers (e.g., mRNA CancerDetect™)."

Pancreatic Enzyme Testing for Acute Pancreatitis

Policy #: AHS – G2153	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/23/22 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Pancreatitis is an inflammation of pancreatic tissue and can be either acute or chronic. Pancreatic enzymes, including amylase, lipase, and trypsinogen can be used to monitor the relative health of the pancreatic tissue. Damage to the pancreatic tissue, including pancreatitis, can result in elevated pancreatic enzyme concentrations whereas depressed enzyme levels are associated with exocrine pancreatic insufficiency (P. A. Banks et al., 2013; Stevens & Conwell, 2020).

II. Related Policies

Policy Number	Policy Title
AHS-M2079	Genetic Testing For Hereditary Pancreatitis

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Measurement of either serum lipase (preferred) **OR** amylase concentration for the initial diagnosis of acute pancreatitis **MEETS COVERAGE CRITERIA** in all patients presenting with signs and symptoms of acute pancreatitis* (please see Note 1).
2. Measurement of either serum lipase **OR** amylase concentration **DOES NOT MEET COVERAGE CRITERIA** in the following situations:

a. As part of an ongoing assessment of therapy for acute pancreatitis; OR

b. In determining the prognosis of pancreatitis; OR

c. In determining the severity or progression of pancreatitis; OR

d. More than once per visit; OR

e. For the diagnosis, prognosis, or severity of chronic pancreatitis; OR

f. As part of ongoing assessment or therapy of chronic pancreatitis

g. In asymptomatic nonpregnant individuals during general exam without abnormal findings

3. Measurement of the following biomarkers for the diagnosis or assessment of acute pancreatitis, prognosis, and/or determination of severity of acute pancreatitis **DOES NOT MEET COVERAGE CRITERIA**:

   a. measurement of both amylase **AND** serum lipase; OR

   b. serum or urine trypsin/trypsinogen/TAP (trypsinogen activation peptide)

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4. Measurement of the following biomarkers for the diagnosis or assessment of acute pancreatitis, prognosis, and/or determination of severity of acute pancreatitis **DOES NOT MEET COVERAGE CRITERIA**:

   a. C-Reactive Protein (CRP)

   b. Interleukin-6 (IL-6)

   c. Interleukin-8 (IL-8)

   d. Procalcitonin

5. Measurement of urinary amylase concentration for the initial diagnosis of acute pancreatitis **DOES NOT MEET COVERAGE CRITERIA** in all patients presenting with signs and symptoms of acute pancreatitis* (please see Note 1)

*Note 1: Acute Pancreatitis Signs and Symptoms (Vege, 2019):

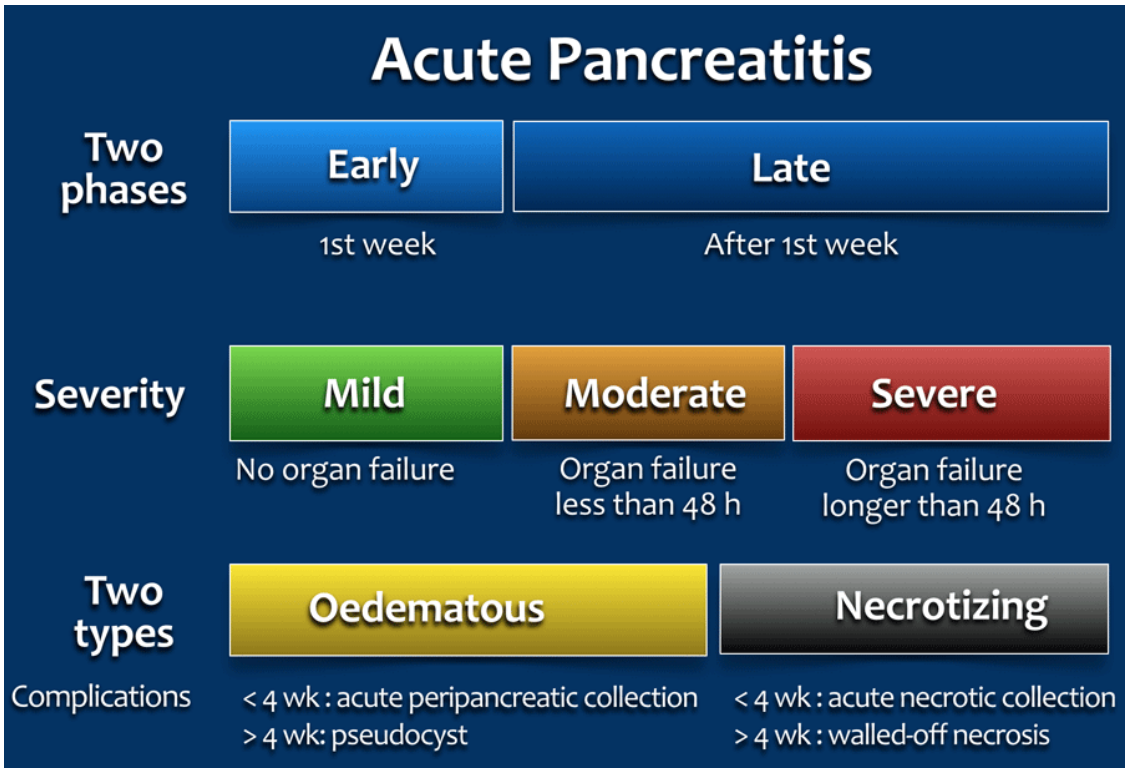
- Persistent, severe epigastric pain (that may be in the right upper quadrant for some patients)
- Nausea
- Vomiting
- "Approximately 5 to 10 percent of patients with acute severe pancreatitis may have painless disease and have unexplained hypotension."
- Tender to palpitation of epigastrium
- Abdominal distention
- Hypoactive bowel sounds

- Fever
- Rapid pulse
- Tachypnea
- Hypoxemia
- Hypotension

IV. Scientific Background

Acute Pancreatitis

Acute pancreatitis (AP) is inflammation of the pancreatic tissue that can range considerably in clinical manifestations. Due to the lack of consensus in diagnosing, characterizing, and treating AP, an international group of researchers and practitioners convened in Atlanta in 1992 to write a clinically based classification system for AP, which is now commonly referred to as the Atlanta convention or Atlanta classification system (Bradley & Iii, 1993). The Atlanta classification system was then revised in 2012 (P. A. Banks et al., 2013). For the diagnosis of AP, two of the three following criteria must be present: "(1) *abdominal pain* consistent with acute pancreatitis (acute onset of a persistent, severe, epigastric pain often radiating to the back); (2) serum *lipase activity (or amylase activity) at least three times greater than the upper limit of normal*; and (3) characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT) and less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography" (italics emphasized by the manuscript's authors) (P. A. Banks et al., 2013). This two-of-three criterion is recommended for diagnostic use by several professional societies (P. Banks & Freeman, 2006; Guidelines, 2013; S. Tenner, Baillie, DeWitt, Vege, & American College of, 2013). AP can be characterized by two temporal phases, early or late, with degrees of severity ranging from mild (with no organ failure) to moderate (organ failure less than 48 hours) to severe (where persistent organ failure has occurred for more than 48 hours). The two subclasses of AP are edematous AP and necrotizing AP. Edematous AP is due to inflammatory edema with relative homogeneity whereas necrotizing AP displays necrosis of pancreatic and/or peripancreatic tissues (P. A. Banks et al., 2013). The figure below from Bollen, Hazewinkel, and Smithuis (2015) outlines the revised Atlanta classification system of AP:



Chronic Pancreatitis

Chronic pancreatitis (CP) is also an inflammation of the pancreatic tissue. The two hallmarks of CP are severe abdominal pain and pancreatic insufficiency (Freedman, 2020). Alcohol-induced chronic pancreatitis (or alcohol pancreatitis) accounts for 60-70% of all cases of CP. CP can be classified in one of nine types (Wilson & Smith, 2015):

- Alcoholic
- Autoimmune (idiopathic)
- Due to trauma
- Inherited factors
- Congenital
- Due to hyperparathyroidism
- Hyperlipidemic
- Due to cystic fibrosis
- Due to protein-energy malnutrition

The endocrine system is comprised of several glands which secrete hormones directly into the bloodstream to regulate many different bodily functions. On the other hand, the exocrine system is comprised of glands which secrete products through ducts, rather than directly into the bloodstream. CP affects both the endocrine and exocrine functions of the pancreas. Fibrogenesis occurs within the pancreatic tissue due to activation of pancreatic stellate cells by toxins (for example, those from chronic alcohol consumption) or cytokines from necroinflammation. Measuring the serum levels of amylase, lipase, and/or trypsinogen is not helpful in diagnosing CP since not every CP patient experiences acute episodes, the relative serum concentrations may be either decreased or unaffected, and the sensitivities of the tests are not enough to distinguish reduced enzyme levels (Witt, Apte, Keim, & Wilson, 2007). The best method to diagnose CP is to histologically analyze a pancreatic biopsy, but this invasive procedure is not always the most practical so "the next best diagnostic methods to demonstrate changes consistent with chronic pancreatitis are computed axial tomography (CT), magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasonography (EUS)" (Wilson & Smith, 2015). Previously, ERCP was commonly used to diagnose CP, but the procedure can cause post-ERCP pancreatitis. Genetic factors are also implicated in CP, especially those related to trypsin activity, the serine protease inhibitor SPINK 1, and cystic fibrosis (Borowitz, Grant, & Durie, 1995; Wilson & Smith, 2015; Witt et al., 2007).

Amylase

Amylase is an enzyme produced predominantly in the salivary glands (s-isoform) and the pancreas (p-isoform or p-isoamylase) and is responsible for the digestion of polysaccharides, cleaving at the internal 1→4 alpha linkage. Up to 60% of the total serum amylase can be of the s-isoform. The concentration of total serum amylase as well as the pancreatic isoenzyme increase following pancreatic injury or inflammation (Basnayake & Ratnam, 2015; Vege, 2020). Even though the serum concentration of the pancreatic diagnostic enzymes, including amylase, lipase, elastase, and immunoreactive trypsin all increase within 24 hours of onset of symptomology, amylase is the first pancreatic enzyme to return to normal levels so the timing of testing is of considerable importance for diagnostic value (Basnayake & Ratnam, 2015; Ventrucci et al., 1987; Yadav, Agarwal, & Pitchumoni, 2002). The half-life of amylase is 12 hours since it is excreted by the kidneys, so its clinical value decreases considerably after initial onset of AP. The etiology of the condition can also affect the relative serum amylase concentration. In up to 50% of AP instances due to hypertriglyceridemia (high blood levels of triglycerides), the serum amylase concentration falls into the normal range, and normal concentrations of amylase has been reported in cases of alcohol-induced AP (Basnayake & Ratnam, 2015; Quinlan, 2014); in fact, one study shows that 58% of the cases of normoamylasemic AP was associated with alcohol use (Clavien et al., 1989). Elevated serum amylase concentrations also can occur in conditions other than AP, including hyperamylasemia (excess amylase in the blood) due to drug exposure (Ceylan, Evrensel, & Önen Ünsalver, 2016; S. Liu et al., 2016), bulimia nervosa (Wolfe, Jimerson, Smith, & Keel, 2011), leptospirosis (Herrmann-Storck et al., 2010), and macroamylasemia (Vege, 2020). Serum amylase levels are often significantly elevated in individuals with bulimia nervosa due to recurrent binge eating episodes (Wolfe et al., 2011).

Macroamylasemia is a condition where the amylase concentration increases due to the formation of macroamylases, complexes of amylase with immunoglobulins and/or polysaccharides.

Macroamylasemia is associated with other disease pathologies, "including celiac disease, HIV infection, lymphoma, ulcerative colitis, rheumatoid arthritis, and monoclonal gammopathy." Suspected macroamylasemia in instances of isolated amylase elevation can be confirmed by measuring the amylase-to-creatinine clearance ratio (ACCR) since macroamylase complexes are too large to be adequately filtered. Normal values range from 3-4% with values of less than 1% supporting the diagnosis of macroamylasemia. ACCR itself is not a good indicator of AP since low ACCR is also exhibited in diabetic ketoacidosis and severe burns (Vege, 2020). Hyperamylasemia is also seen in other extrapancreatic conditions, such as appendicitis, salivary disease, gynecologic disease, extra-pancreatic tumors, and gastrointestinal disease (Terui et al., 2013; Vege, 2020). Gullo's Syndrome (or benign pancreatic hyperenzymemia) is a rare condition that also exhibits high serum concentrations of pancreatic enzymes without showing other signs of pancreatitis (Kumar, Ghosh, Tandon, & Sahoo, 2016). No correlation has been found between the concentration of serum amylase and the severity or prognosis of AP (Lippi, Valentino, & Cervellin, 2012).

Urinary amylase and peritoneal amylase concentrations can also be measured. Rompianesi et al. (2017) reviewed the use of urinary amylase and trypsinogen as compared to serum amylase and serum lipase testing. The authors note that "with regard to urinary amylase, there is no clear-cut level beyond which someone with abdominal pain is considered to have acute pancreatitis." Three studies regarding urinary amylase were reviewed —each with 134-218 participants—and used the hierarchical summary receiver operating characteristics curve (HSROC) analysis to compare the accuracy of the studies. Results showed that "the models did not converge" and the authors concluded that "we were therefore unable to formally compare the diagnostic performance of the different tests" (Rompianesi et al., 2017).

Another study investigated the use of peritoneal amylase concentrations for diagnostic measures and discovered that patients with intra-abdominal peritonitis had a mean peritoneal amylase concentration of 816 U/L (142-1746 U/L range), patients with pancreatitis had a mean concentration of 550 U/L (100-1140 U/L range), and patients with other "typical infectious peritonitis" had a mean concentration of 11.1 U/L (0-90 U/L range). Conclusions state "that peritoneal fluid amylase levels were helpful in the differential diagnosis of peritonitis in these patients" and that levels >100 U/L "differentiated those patients with other intra-abdominal causes of peritonitis from those with typical infectious peritonitis" (Burkart, Haigler, Caruana, & Hylander, 1991). The authors do not state if intraperitoneal amylase is specifically useful in diagnosing AP.

Lie et al. (2021) conducted a retrospective cohort study to evaluate whether serum amylase and lipase could serve as a biomarker to predict pancreatic injury in 79 critically ill children who died of different causes. Through autopsy investigation, the subjects were divided into pancreatic injury group and non-pancreatic injury group. 41 patients (51.9%) exhibited pathological changes of pancreatic injury. Levels of lactate, erythrocyte sedimentation rate, alanine transaminase, aspartate transaminase, and troponin-I in the pancreatic injury group were significantly higher than that in the noninjury group. "Multivariable logistic regression analysis showed that serum amylase, serum lipase, and septic shock were significantly associated with the occurrence rate of pancreatic injury." Therefore, the authors conclude that "serum amylase and lipase could serve as independent biomarkers to predict pancreatic injury in critically ill children (P. Liu et al., 2021)."

In a prospective case control study, Judal (2022) investigated urinary amylase levels for diagnosis of acute pancreatitis. One major challenge with measurement of serum amylase is its short half-life which returns to normal levels within a short period of time. This study enrolled 100 patients (50 healthy and 50 with acute pancreatitis) who were measured for serum amylase, serum lipase, and urinary amylase. There was a statistically significant increase in the serum amylase, lipase, and urinary amylase mean values of patients with AP. "Serum amylase had the highest sensitivity (100%) and serum lipase had the highest specificity (96.53%). The sensitivity and specificity of urinary amylase was found to be 97.25% and 91.47% respectively" (Judal, 2022). The authors conclude that urinary amylase is a convenient and sensitive test for diagnosis.

Lipase (Pancreatic Lipase or Pancreatic Triacylglycerol Lipase)

Pancreatic lipase or triacylglycerol lipase (herein referred to as "lipase") is an enzyme responsible for hydrolyzing triglycerides to aid in the digestion of fats. Like amylase, lipase concentration increases shortly after pancreatic injury (within 3-6 hours). However, contrary to amylase, serum lipase concentrations remain elevated for 1-2 weeks after initial onset of AP since lipase can be reabsorbed by the kidney tubules (Lippi et al., 2012). Moreover, the pancreatic lipase concentration is 100-fold higher than the concentration of other forms of lipases found in other tissues such as the duodenum and stomach (Basnayake & Ratnam, 2015). Both the sensitivity and the specificity of lipase in laboratory testing of AP are higher than that of amylase (Yadav et al., 2002). A study by Coffey, Nightingale, and Ooi (2013) found "an odds ratio of 7.1 (95% confidence interval 2.5-20.5; P<0.001) for developing severe AP" in patients ages 18 or younger when the serum lipase concentration is at least 7-fold higher than upper limit of normal. However, in general, elevated serum lipase concentration is not used to determine the severity or prognosis of AP (Ismail & Bhayana, 2017). Hyperlipasemia can also occur in other conditions such as Gullo's Syndrome (Kumar et al., 2016). The use of lipase to determine etiology of AP is of debate. A study by Levy et al. (2005) reports that lipase alone cannot be used to determine biliary cause of AP whereas other studies have indicated that a ratio of lipase-to-amylase concentrations ranging from 2:1 to more than 5:1 can be indicative of alcohol-induced AP (Gumaste, Dave, Weissman, & Messer, 1991; Ismail & Bhayana, 2017; Pacheco & Oliveira, 2007; S. M. Tenner & Steinberg, 1992).

The review by Ismail and Bhayana (2017) included a summary table (Table 1 below) comparing various studies concerning the use of amylase and lipase for diagnosis of AP as well as a table (Table 2 below) comparing the cost implication of the elimination of double-testing for AP.

Table 1: Summary of numerous studies comparing lipase against amylase (URL – Upper Limit of Reference interval, AP – Acute Pancreatitis).

Design and reference	Participant (patients with abdominal pain/AP)	Threshold	Results		Conclusion
			Serum lipase	Serum amylase	
Prospective study [56]	384/60	Two times URL	Diagnostic accuracy and efficiency are > 95% for both		No difference between amylase

					and lipase in diagnosing AP
Prospective study [57]	306/48	Serum lipase > 208 U/L Serum amylase > 110 U/L	92% sensitivity 87% specificity 94% Diagnostic accuracy	93% sensitivity 87% specificity 91% Diagnostic accuracy	Both tests are associated with AP, but serum lipase is better than amylase
Prospective study [58]	328/51	Serum lipase: > 208 U/L (Day 1) > 216 U/L (Day 3) Serum amylase: > 176 U/L > 126 U/L (Day 3)	Day 1: 64 % Sensitivity 97% Specificity Day 3: 55% Sensitivity 84% Specificity	Day 1: 45 % Sensitivity 97% Specificity Day 3: 35% Sensitivity 92% Specificity	Serum lipase is better at diagnosing early and late AP
Retrospective study [63]	17,531/320 *49 had elevated lipase only	Serum lipase > 208 U/L Serum amylase > 114 U/L	90.3% Sensitivity 93.6% Specificity	78.7% Sensitivity 92.6% Specificity	Serum lipase is more accurate marker for AP
Cohort study [2]	1,520/44	Three times URL	64% Sensitivity 97% Specificity	50% Sensitivity 99% Specificity	Serum lipase is preferable to use in comparison to amylase alone or both tests
Retrospective study [59]	3451/34 *33 patients had elevated amylase and 50 had elevated lipase only	Three or more times URL	95.5% Sensitivity 99.2% Specificity	63.6% Sensitivity 99.4% Specificity	Both enzymes have accuracy, but lipase is more sensitive than amylase
Cohort study [60]	151/117 *6 patients with gallstone-induced and 5 patients with alcohol-induced AP had elevated lipase only	Three times URL	96.6% Sensitivity 99.4% Specificity	78.6% Sensitivity 99.1% Specificity	Lipase is more sensitive in diagnosing AP and using it alone would present a substantial cost saving on health care system
Prospective study [61]	476/154 *58 patients had a normal amylase level	Three times URL	91% Sensitivity 92% Specificity	62% Sensitivity 93% Specificity	Lipase is more sensitive than amylase and should replace amylase in diagnosis of AP

Cohort study [62]	50/42 *8 patients had elevated lipase only	Three times URL	100% Sensitivity	78.6% Sensitivity	Lipase is a better choice than amylase in diagnosis of AP

This table is a list of individual studies examining the specificity and sensitive of serum lipase and serum amylase in diagnosing AP. In each of the listed studies except one, the authors concluded that serum lipase is better than serum amylase for AP. The only outlier used a lower threshold in considering enzyme elevation; in particular, two times the upper limit of reference interval (URL) was used whereas the Atlanta classification system recommends at least three times the URL to determine enzyme elevation (Ismail & Bhayana, 2017).

Table 2: Summary of studies exploring the cost implication associated with eliminating amylase test.

Design and Reference	Costs	Volume of test	Results
Cohort study (UK) [2]	Amylase costs £1.94 Lipase cost £2.50	1383 request for 62 days costing £6136 for both tests	Testing lipase only will result in cost saving
Cohort study (UK) [60]	Single amylase or lipase cost about £0.69 each Cost of both measured together were £0.99.	2979 requests costing £2949.21	Measuring lipase would save health care system an estimate of £893.70 per year
Prospective study (US) [71]	Patients charged $35 for either lipase or amylase	618 co-ordered both lipase and amylase	Amylase test was removed from common order sets in the electronic medical record Reduced the co-ordering of lipase and amylase to 294 Overall saving of $135,000 per year

This table specifically outlines studies that compared the financial cost of the serum amylase and serum lipase tests for diagnosing AP. All three studies show cost savings if only lipase concentration is used. In fact, one study by researchers in Pennsylvania resulted in the removal of the amylase test "from common order sets in the electronic medical record" (Ismail & Bhayana, 2017).

Furey, Buxbaum, and Chambliss (2020) compared amylase and lipase ordering patterns for patients with AP. A total of 438 individuals were included in this study. The researchers noted that "All patients had at least one lipase ordered during their admission, and only 51 patients (12%) had at least one amylase ordered. On average, lipase was elevated 5 times higher above its respective upper reference limit than amylase at admission (Furey et al., 2020)." Further, patients undergoing a laparoscopic cholecystectomy (gallbladder removal) were more likely to have amylase ordered. These results showed that in 88% of patients with AP, amylase measurement was not necessary; moreover, "Of patients for whom amylase was ordered, it was common for these patients to be those referred to surgical procedures, possibly because amylase normalization may be documented faster than that of lipase (Furey et al., 2020)."

In a retrospective cross-sectional study by El Halabi et al. (2019), the clinical utility and economic burden of routine serum lipase examination in the emergency department was observed. From 24,133 adult patients admitted within a 12-month period, serum lipase levels were ordered for 4,976 (20.6%) patients. Of those 614 (12.4%) who had abnormal lipase levels, 130 of those patients were above the diagnostic threshold for acute pancreatitis (>3 times the ULN) and 75 patients had confirmed diagnosis of acute pancreatitis. 1,890 patients had normal no abdominal pain or history of acute pancreatitis, but 251 of these patients were tested for lipase levels, leading to a total cost of $51,030. These results triggered unneeded cross-sectional abdominal imaging in 61 patients and unwarranted gastroenterology consultation in 3 patients, leading to an additional charge of $28,975. The authors conclude that "serum lipase is widely overutilised in the emergency setting resulting in unnecessary expenses and investigations (El Halabi et al., 2019)."

Liu et al. (2021) studied the use of serum amylase and lipase for the prediction of pancreatic injury in critically ill children admitted to the PICU. 79 children who died from different cases were studied from autopsy and it was found that 41 of these patients had pathological signs of pancreatic injury. Multivariable logistic regression analysis showed that serum amylase, serum lipase, and septic shock were significantly associated with the occurrence rate of pancreatic injury. Serum amylase was measured with 53.7% sensitivity, 81.6% specificity, cut off value of 97.5, and AUC of 0.731. Serum lipase was measured with 36.6% sensitivity, 92.1% specificity, cut off value of 61.1, and AUC of 0.727. The authors conclude that “serum amylase and lipase could serve as independent biomarkers to predict pancreatic injury in critically ill children” (P. Liu et al., 2021).

Trypsin/Trypsinogen/TAP

Trypsin is a protease produced by the pancreatic acinar cells. It is first synthesized in its zymogen form, trypsinogen, which has its N-terminus cleaved to form the mature trypsin. Pancreatitis can result in blockage of the release of the proteases while their synthesis continues. This increase in both intracellular trypsinogen and cathepsin B, an enzyme that can cleave the trypsinogen activation peptide (TAP) from the zymogen to form mature trypsin, results in a premature intrapancreatic activation of trypsin. This triggers a release of both trypsin and TAP extracellularly into the serum and surrounding peripancreatic tissue. Due to the proteolytic nature of trypsin, this response can result in degradation of both the pancreatic and peripancreatic tissues (necrotizing AP) (Vege, 2021; Yadav et al., 2002). Trypsin activity “is critical for the severity of both acute and chronic pancreatitis” (Zhan et al., 2019). When the intracellular activity of trypsin escalates, an increase is also reflected in the number of pancreatitis cases overall, as well as in the severity of these cases(Sendler & Lerch, 2020).

Since trypsinogen is readily excreted, a urine trypsinogen-2 dipstick test has been developed (Actim Pancreatitis test strip from Medix Biochemica), which has a reported specificity of 85% for severe AP within 24 hours of hospital admission (Lempinen et al., 2001). Another study reported that the trypsinogen-2 dipstick test has a specificity of 95% and a sensitivity of 94% for AP, which is higher than a comparable urine test for amylase (Kemppainen et al., 1997). As of 2020, the FDA has not approved the use of the trypsinogen-2 dipstick test for the detection or diagnosis of AP. Clinical trials are underway in the United States (Eastler, 2017). The use of TAP for either a diagnostic or prognostic tool is of debate (Lippi et al., 2012).

The study by Neoptolemos et al. (2000) reported that a urinary TAP assay had a 73% specificity for AP. However, another study using a serum TAP methodology reported a 23.5% sensitivity and 91.7% specificity for AP and concluded that "TAP is of limited value in assessing the diagnosis and the severity of acute pancreatic damage" (Pezzilli et al., 2004).

Yasuda et al. (2019) completed a multicenter study in Japan which measured the usefulness of the rapid urinary trypsinogen-2 dipstick test and levels of urinary trypsinogen-2 and TAP concentration as prognostic tools for AP. A total of 94 patients participated in this study from 17 medical institutions between April 2009 and December 2012. The researchers determined that "The trypsinogen-2 dipstick test was positive in 57 of 78 patients with acute pancreatitis (sensitivity, 73.1%) and in 6 of 16 patients with abdominal pain but without any evidence of acute pancreatitis (specificity, 62.5%) (Yasuda et al., 2019)." Further, both TAP and urinary trypsinogen-2 levels were significantly higher in patients with extra-pancreatic inflammation. The authors concluded that the urinary trypsinogen-2 dipstick test is a useful tool for AP diagnoses.

Simha at al. (2021) studied the utility of POC urine trypsinogen dipstick test for diagnosing AP in an emergency unit. Urine trypsinogen dipstick test (UTDT) was performed in 187 patients in which 90 patients had AP. UTDT was positive in 61 (67.7%) of the 90 AP patients. In the 97 non pancreatitis cases, UTDT was positive in 9 of those cases (9.3%). The sensitivity and specificity of UTDT for acute pancreatitis was 67.8% and 90.7%, respectively. The authors conclude that although it is a great and convenient possibility as a POC test, "the low sensitivity of UTDT could be a concern with its routine use" (Simha et al., 2021).

Other Biochemical Markers (CRP, Procalcitonin, IL-6, IL-8)

AP results in the activation of the immune system. Specific markers including C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6), and interleukin-8 (IL-8) have been linked to AP (Toouli et al., 2002; Vege, 2019; Yadav et al., 2002). CRP is a nonspecific marker for inflammation that takes 48-72 hours to reach maximal concentration after initial onset of AP but is reported to have a specificity of 93% in detecting pancreatic necrosis. CRP can be used in monitoring the severity of AP; however, imaging techniques, including CT, and evaluative tools, such as the APACHE-II (acute physiology and chronic health evaluation) test, are preferred methods (Guidelines, 2013; Quinlan, 2014).

Procalcitonin is the inactive precursor of the hormone calcitonin. Like CRP, procalcitonin has been linked to inflammatory responses, especially in response to infections and sepsis. Procalcitonin levels are elevated in AP and are significantly elevated (≥3.5 ng/mL for at least two consecutive days) in cases of AP associated with multiorgan dysfunction syndrome (MODS) (Rau et al., 2007). Moreover, the elevated procalcitonin levels decrease upon treatment for AP; "however, further research is needed in order to understand how these biomarkers can help to monitor inflammatory responses in AP" (Simsek et al., 2018).

The concentration of inflammatory cytokines IL-6 and IL-8 become elevated in AP with a maximal peak within the first 24 hours after initial onset of AP (Yadav et al., 2002). One study by Jakkampudi et al. (2017) shows that IL-6 and IL-8 are released in a time-dependent manner after injury to the pancreatic acinar cells. This, in turn, activated the peripheral blood mononuclear cells (PBMCs), which propagate

acinar cell apoptosis that results in further release of cytokines to increase the likelihood of additional cellular damage.

A study conducted by Khanna et al. (2013) compares the use of biochemical markers, such as CRP, IL-6, and procalcitonin, in predicting the severity of AP and necrosis to that of the clinically used evaluative tools, including the Glasgow score and APACHE-II test. Their results indicate that CRP has a sensitivity and specificity of 86.2% and 100%, respectively, for severe AP and a sensitivity and specificity of 100% and 81.4%, respectively, for pancreatic necrosis. These scores are better than those reported for the clinical evaluative tools (see table below). IL-6 also shows an increase in both sensitivity and specificity; however, the values for procalcitonin are considerably lower than either CRP or IL-6 in all parameters (Khanna et al., 2013).

Data from *(Khanna et al., 2013)*	**Severe AP**		**Pancreatic necrosis**	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
Glasgow	71.0	78.0	64.7	63.6
APACHE-II	80.6	82.9	64.7	61.8
CRP	86.2	100	100	81.4
IL-6	93.1	96.8	94.1	72.1
Procalcitonin	86.4	75.0	78.6	53.6

Another study by Hagjer and Kumar (2018) compared the efficacy of the bedside index for severity in acute pancreatitis (BISAP) scoring system to CRP and procalcitonin shows that CRP is not as accurate for prognostication as BISAP. BISAP has AUCs for predicting severe AP and death of 0.875 and 0.740, respectively, as compared to the scores of CRP (0.755 and 0.693, respectively). Procalcitonin, on the other hand, had values of 0.940 and 0.769 for predicting severe AP and death, respectively. The authors concluded that it "is a promising inflammatory marker with prediction rates similar to BISAP" (Hagjer & Kumar, 2018).

Li, Wang, Cai, and Liu (2018) completed a meta-analysis to determine the relationship between high mobility group box 1 (HMGB1), interleukin-6 (IL-6) and AP. HMGB1 protein is a nuclear protein with several different purposes depending on its location (Yang, Wang, Chavan, & Andersson, 2015). These researchers analyzed data from 27 different studies comprised of 1908 of participants (896 with mild AP, 700 with severe AP and 312 healthy controls). Overall, serum HMGB1 and IL-6 levels were higher in patients with both severe and mild AP compared to controls; further, and serum HMGB1 and IL-6 levels were significantly higher in patients with severe AP than mild AP (Li et al., 2018). The authors concluded that serum HMGB1 and IL-6 levels "might be used as effective indicators for pancreatic lesions as well as the degree of inflammatory response" and that both HMGB1 and IL-6 are closely correlated with pancreatitis severity.

Tian et al. (2020) studied the diagnostic value of C-reactive protein (CRP), procalcitonin (PCT), IL-6, and lactate dehydrogenase (LDH) in patients with severe acute pancreatitis. 153 patients were divided into the mild acute pancreatitis group (81) and severe pancreatitis group (72). Significant differences in the values of these enzymes were found between both groups. The sensitivity, specificity, and AUC were determined as seen in the chart below. The AUC of combined detection of CRP, PCT, IL-6 and LDH was 0.989. The authors conclude that "the combined detection of CRP, PCT, IL-6 and LDH has a high diagnostic value for judging the severity of acute pancreatitis (Tian et al., 2020)."

Enzyme	Sensitivity	Specificity	AUC
CRP	55.6%	73%	0.637
PCT	77.8%	94%	0.929
IL-6	80.2%	85%	0.886
LDH	82.7%	96%	0.919

In a retrospective cohort study, Wei investigated the predictive value of serum cholinesterase (ChE) in the mortality of acute pancreatitis. 692 patients were enrolled in the study and were divided into the ChE-low group (378 patients) or ChE-normal group (314 patients). Mortality was significantly different in two groups (10.3% in ChE-low vs. 0.0% ChE- normal) and organ failure also differed (46.6% ChE-low vs. 8.6% ChE-normal). The area under the curve of serum ChE was 0.875 and 0.803 for mortality and organ failure, respectively. The authors conclude that "lower level of serum ChE was independently associated with the severity and mortality of AP" (Wei et al., 2022).

V. Guidelines and Recommendations

International Association of Pancreatology (IAP) and the American Pancreatic Association (APA)

In 2012, a joint conference between the International Association of Pancreatology (IAP) and the American Pancreatic Association (APA) convened to address the guidelines for the management of acute pancreatitis. This conference made their recommendations using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system. The guidelines are detailed with 38 recommendations covering 12 different topics, ranging from diagnosis to predicting severity of disease to timing of treatments. As concerning the diagnosis and etiology of AP, the associations conclude with "GRADE 1B, strong agreement" that the definition of AP follow the Atlanta classification system where at least two of the following three criteria are evident—the clinical manifestation of upper abdominal pain, the laboratory testing of serum amylase or serum lipase where levels are >3 times the upper limit of normal values, and/or the affirmation of pancreatitis using imaging methods. They specifically did not include the trypsinogen-2 dipstick test in their recommendations "because of its presumed limited availability." One question addressed by the committee was the continuation of oral feeding being withheld for patients until the lab serum tests returned within normal values. With a GRADE 2B, strong agreement finding, they conclude that "it is not necessary to wait until pain or laboratory abnormalities completely resolve before restarting oral feeding." No specific discussion on the preference of either

serum amylase or lipase is included within the guidelines as well as no discussion of the use of either serum test beyond initial diagnosis of AP (i.e., no continual testing for disease monitoring is included). Furthermore, no discussion concerning the use of urinary or peritoneal amylase concentrations for AP.

With regards to CRP and/or procalcitonin, the IAP/APA does not address the topic in any detail. As part of their recommendation (GRADE 2B) concerning the best score or marker to predict the severity of AP, they state "that there are many different predictive scoring systems for acute pancreatitis…, including single serum markers (C-reactive protein, hematocrit, procalcitonin, blood urea nitrogen), but none of these are clearly superior or inferior to (persistent) SIRS", which is Systemic inflammatory response syndrome. Moreover, in response to their recommendation for admission to an intensive care unit in AP (GRADE 1C), they state that "the routine use of single markers, such as CRP, hematocrit, BUN or procalcitonin alone to triage patients to an intensive care setting is not recommended."

American Gastroenterological Association (AGA)

The Clinical Practice and Economics Committee (CPEC) of the American Gastroenterological Association (AGA) Institute released the AGA Institute Medical Position Statement on Acute Pancreatitis as approved by the AGA Institute Governing Board in 2007 to address differences in the recommendations of various national and international societies concerning AP. Within their recommendations, they address the necessity of timeliness in the applicability of serum amylase and/or serum lipase testing. Per their recommendations, either serum amylase or serum lipase should be tested within 48 hours of admission. AP is consistent with amylase or lipase levels greater than 3 times the upper limit of the normal value. They specifically state that the "elevation of lipase levels is somewhat more specific and is thus preferred." The AGA guidelines do not address the use of either urinary or peritoneal concentrations of amylase in AP. Also, any patient presenting symptoms of unexplained multiorgan failure or systemic inflammatory response syndrome should be tested for a possible AP diagnosis. Concerning etiology of the phenotype, they suggest that upon admission, "all patients should have serum obtained for measurement of amylase or lipase level, triglyceride level, calcium level, and liver chemistries." Invasive evaluation, such as endoscopic retrograde cholangiopancreatography (ERCP), should be avoided for patients with a single occurrence of AP. The only mention of CRP in their guidelines is in the section concerning the severity (and not the diagnosis of) AP. "Laboratory tests may be used as an adjunct to clinical judgment, multiple factor scoring systems, and CT to guide clinical triage decisions. A serum C-reactive protein level >150 mg/L at 48 hours after disease onset is preferred."

In 2018, the AGA published guidelines on the initial management of AP. These guidelines state that "The diagnosis of AP requires at least 2 of the following features: characteristic abdominal pain; biochemical evidence of pancreatitis (ie, amylase or lipase elevated >3 times the upper limit of normal); and/or radiographic evidence of pancreatitis on cross-sectional imaging (Crockett et al., 2018)."

American College of Gastroenterology (ACG)

The ACG released guidelines concerning AP in both 2006 and 2013. Both sets of guidelines recommend the use of the Atlanta classification system regarding the threshold of either serum amylase or serum lipase levels in the diagnosis of AP (i.e., greater than three times the upper limit of normal range). Both

sets of guidelines state that the standard diagnosis is meeting at least two of the three criteria as stated in the revised Atlanta classification system.

The 2006 guidelines discuss the differences between serum amylase and lipase in greater detail. First, although both enzymes can be elevated in AP, the sensitivity and half-life of lipase are more amenable for diagnosis since the levels of lipase remain elevated longer than those of amylase. These guidelines also make note that "it is usually not necessary to measure both serum amylase and lipase" and that "the daily measurement of serum amylase or lipase after the diagnosis of acute pancreatitis has limited value in assessing the clinical progress of the illness." These guidelines discuss the possibility of elevated amylase levels due to causes other than AP, including but not limited to macroamylasemia, whereas the serum levels of lipase are unaffected by these conditions.

The 2013 guidelines do not explicitly state a preference of the serum lipase over serum amylase test in the diagnosis of AP. They also state that lipase levels can be elevated in macrolipasemia as well as certain nonpancreatic conditions, "such as renal disease, appendicitis, cholecystitis, and so on." Neither set of guidelines address the use of either urinary or peritoneal amylase in AP. The 2006 guidelines list other diagnostic tests, including the trypsin/trypsinogen tests as well as serum amyloid A and calcitonin but do not address them further given their limited availability at that time whereas the 2013 guidelines state that, even though other enzymes can be used for diagnostics, "none seems to offer better diagnostic value than those of serum amylase and lipase." They even state that "even the acute-phase reactant C-reactive protein (CRP) the most widely studied inflammatory marker in AP, is not practical as it takes 72h to become accurate."

UK Working Party on Acute Pancreatitis

The UK Working Party on Acute Pancreatitis consists of a consortium of the British Society of Gastroenterology, Association of Surgeons of Great Britain and Ireland, Pancreatic Society of Great Britain and Ireland, and the Association of Upper GI Surgeons of Great Britain and Ireland. The recommendation by the UK Working Party is that "although amylase is widely available and provides acceptable accuracy of diagnosis, where lipase estimation is available it is preferred for the diagnosis of acute pancreatitis (recommendation grade A)." One contrast of the guidelines of the UK Working Party as compared to other professional societies is the relative threshold of the serum concentrations of pancreatic enzymes. Rather than use the >3 times the upper limit of the normal concentrations of either amylase or lipase as stated in the Atlanta classification system, the UK Working Party's guidelines state that AP diagnosis "should not rely on arbitrary limits of values 3 or 4 times greater than normal, but values should be interpreted in light of the time since the onset of abdominal pain." These elevated serum levels as well as the clinical abdominal symptoms are the "cornerstones of diagnosis." They do not address the frequency of serum enzyme testing or the use of trypsin/trypsinogen-based tests, urinary amylase, or peritoneal amylase.

American Board of Internal Medicine (ABIM), American Society for Clinical Pathology (ASCP) and Choosing Wisely

The American Board of Internal Medicine (ABIM) Foundation oversees the Choosing Wisely initiative where various professional societies can publish recommendations. The American Society for Clinical

Pathology (ASCP) released a series of recommendations via Choosing Wisely beginning in 2013. In 2016, the ASCP released a recommendation clearly stating, "Do not test for amylase in cases of suspected acute pancreatitis. Instead, test for lipase…. Current guidelines and recommendations indicate that lipase should be preferred over total and pancreatic amylase for the initial diagnosis of acute pancreatitis and that the assessment should not be repeated over time to monitor disease prognosis." The ASCP also states that performing both lipase and amylase tests are not cost-effective given "marginally improving diagnostic efficiency compared to either marker alone." The ASCP recommendation does not mention any trypsin- or trypsinogen-based methodologies.

In 2020, the ASCP, along with Choosing Wisely and the ABIM Foundation, published a brochure titled *Thirty Things Physicians and Patients Should Question.* This brochure includes the following recommendation:

"Do not test for amylase in cases of suspected acute pancreatitis. Instead, test for lipase.

Amylase and lipase are digestive enzymes normally released from the acinar cells of the exocrine pancreas into the duodenum. Following injury to the pancreas, these enzymes are released into the circulation. While amylase is cleared in the urine, lipase is reabsorbed back into the circulation. In cases of acute pancreatitis, serum activity for both enzymes are greatly increased.

Serum lipase is now the preferred test due to its improved sensitivity, particularly in alcohol-induced pancreatitis. Its prolonged elevation creates a wider diagnostic window than amylase. In acute pancreatitis, amylase can rise rapidly within 3–6 hours of the onset of symptoms and may remain elevated for up to five days. Lipase, however, usually peaks at 24 hours with serum concentrations remaining elevated for 8–14 days. This means it is far more useful than amylase when the clinical presentation or testing has been delayed for more than 24 hours.

Current guidelines and recommendations indicate that lipase should be preferred over total and pancreatic amylase for the initial diagnosis of acute pancreatitis and that the assessment should not be repeated over time to monitor disease prognosis. Repeat testing should be considered only when

the patient has signs and symptoms of persisting pancreatic or peripancreatic inflammation, blockage of the pancreatic duct or development of a pseudocyst. Testing both amylase and lipase is generally discouraged because it increases costs while only marginally improving diagnostic efficiency compared to either marker alone (ASCP, 2020)."

North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Pancreas Committee (NASPGHAN)

NASPGHAN states that the primary biomarkers used to diagnose AP are serum lipase and amylase and note that "a serum lipase or amylase level of at least 3 times the upper limit of normal is considered consistent with pancreatitis." Further, NASPGHAN acknowledges that other biomarkers for diagnosis and management of AP have been investigated, but none are prominent and "many have yet to be validated for general clinical use."

American Pancreatic Association (APA)

The APA acknowledges the utility of the measure of serum lipase and amylase for assessing pancreatic function "especially if they have a low value."

All societies reviewed prefer the use of serum lipase over serum amylase in the diagnosis of AP based on the higher sensitivity and selectivity of lipase. No consensus concerning the diagnostic threshold is reached between all of the societies where some use a threshold based on the Atlanta classification system, some do not specify a threshold, and one consortium recommends a time-based value system. A table adapted from the review by Lippi et al. (2012) summarizing the recommendations from various societies is below. This table has also been modified and updated with recent guidelines.

Table 2. Synthesis of available guidelines and recommendations for laboratory testing in the diagnosis of acute pancreatitis

Organization(s)	Preferred biomarker	Diagnostic threshold
Société Nationale Française de Gastro-Entérologie	Lipase	≥ 3 times the URL [upper limit of reference]
Japanese Society of Emergency Abdominal Medicine	Lipase	Not set
British Society of Gastroenterology; Association of Surgeons of Great Britain and Ireland, Pancreatic Society of Great Britain and Ireland, Association of Upper GI Surgeons of Great Britain and Ireland	Lipase	Value interpreted according to the time since the onset of symptoms
American of Gastroenterology	Lipase	≥ 2 to ≥ 4 times the URL
American Gastroenterological Association	Lipase	≥ 3 times the URL
American of Family Physicians	Lipase	Not set
Japanese Ministry of Health, Labour, and Welfare	Lipase	Not set
Working Group of the Italian Association for the Study of the Pancreas	Lipase	Not set
World Society of Emergency Surgery*	Lipase	3 times the URL
North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Pancreas Committee*	Amylase or Lipase	3 times the URL
American Board of Internal Medicine (ABIM), American Society for Clinical Pathology (ASCP) and Choosing Wisely*	Lipase	Not stated
UK Working Party on Acute Pancreatitis*	Lipase	should not rely on arbitrary limits of values 3 or 4 times greater than normal
American College of Gastroenterology*	Amylase or Lipase	≥ 3 times the URL
International Association of Pancreatology and the American Pancreatic Association*	Amylase or Lipase	≥ 3 times the URL

* This information was not part of the original table and was added to reference additional guidelines

The World Society of Emergency Surgery (WSES)

The WES published guidelines in 2019 on the management of severe AP. These guidelines were determined by a panel of experts in 2018. The following statement was issued: "The cut-off value of serum amylase and lipase is normally defined to be three times the upper limit." Further, it is noted that

"Collectively, serum lipase is considered a more reliable diagnostic marker of AP than serum amylase. No single test shows optimal diagnostic accuracy, but most current guidelines and recommendations indicate that lipase should be preferred over total and p-amylase. The main reasons supporting lipase over both types of amylase for the diagnosis of acute pancreatitis include higher sensitivity and larger diagnostic window (Leppaniemi et al., 2019)."

American Psychiatric Association (APA)

The APA published guidelines in 2006 for the treatment of patients with eating disorders. As shown below, these guidelines mention pancreatitis in several different tables throughout the document (table 5, table 6 and table 7).

- Table 5. Physical complications of Anorexia Nervosa
  - Regarding gastrointestinal laboratory test results, the APA (2006) states the following: "Liver function and associated tests: Occasionally abnormal liver function test results, increased serum amylase in purging patients; abnormal results rarely due to pancreatitis"
- Table 6: Physical complications of Bulimia Nervosa
  - Regarding gastrointestinal signs, the APA (2006) states the following: "Enlarged salivary glands, occasional blood-streaked vomitus; in vomiters, possibly gastritis, esophagitis, gastroesophageal erosions, esophageal dysmotility patterns (including gastroesophageal reflux and, rarely, Mallory-Weiss [esophageal] or gastric tears), increased rates of pancreatitis; in chronic laxative abusers, possibly colonic dysmotility or melanosis coli"
  - Regarding gastrointestinal laboratory test results, the APA (2006) states the following: "Serum amylase: Increased serum amylase (if fractionation is available, usually salivary gland isoenzymes); increased pancreatic amylase (rare), possibly indicating laxative abuse or other causes for pancreatic inflammation or pancreatitis"
  - Table 7: Laboratory Assessments for Patients with Eating Disorders
  - Regarding nonroutine assessments, the APA (2006) has included "Serum amylase (fractionated for salivary gland isoenzyme if available to rule out pancreatic involvement)"

Academy for Eating Disorders (AED) Medical Care Standards Committee (AED)

The AED has published a guide to medical care for eating disorders. A table is included in these guidelines which is titled *Diagnostic Tests Indicated for All Patients with a Suspected ED* [eating disorder]. In a subcategory, titled *Additional Diagnostic Tests to Consider*, these guidelines mention pancreatic enzymes (amylase and lipase), stating that amylase could increase due to vomiting and pancreatitis, and lipase could increase due to pancreatitis.

The Canadian Journal of Surgery

The Canadian Journal of Surgery released clinical practice guidelines on the management of acute pancreatitis. The following recommendations were made (Greenberg et al., 2016):

Guideline Recommendation	Strength of Evidence
1. "A serum lipase test should be performed in all patients with a suspected diagnosis of acute pancreatitis. A 3-fold elevation of serum lipase from the upper limit of normal is required to make the diagnosis of acute pancreatitis	Strong
2. C-reactive protein should be assessed at admission and daily for the first 72 hours after admission	Weak
3. A serum C-reactive protein (CRP) level of 14 286 nmol/L (150 mg/dL) or greater at baseline or in the first 72 hours is suggestive of severe acute pancreatitis and is predictive of a worse clinical course. Thus, CRP should be assessed at admission and daily for the first 72 hours after admission."	Strong

The American Association for Clinical Chemistry (AACC)

The American Association for Clinical Chemistry released recommendations for amylase testing in diagnosis and management of acute pancreatitis. The AACC provides the following recommendations:

1. "For diagnosis and management of acute pancreatitis, do not order the amylase test if serum lipase test is available.
2. The amylase test is not specific for pancreatitis and may be elevated due to other, non-pancreatic causes (such as acute cholecystitis, inflammatory bowel disease, intestinal obstruction, certain cancers, salivary disease, macroamylasemia, etc.)
3. Consider ordering this test when serum lipase is not available as a stat test and the patient presents with a sudden onset of abdominal pain with nausea and vomiting, fever, hypotension, and abdominal distension.
4. Testing both amylase and lipase should be discouraged because it increases costs while only marginally improving diagnostic efficiency compared to lipase alone (AACC, 2020)."

Chinese Pancreatic Surgery Association, Chinese Society of Surgery, Chinese Medical Association

The following associations discusses guidelines for the diagnosis of acute pancreatitis in China. "The diagnostic criteria of AP include (1) persistent upper abdominal pain, (2) concentration of serum amylase and/or lipase at least 3 times higher than the normal upper limit, and (3) abdominal imaging findings suggestive of AP. AP can be diagnosed if 2 of the above 3 criteria are met." The guidelines also state that "Laboratory examination results show elevated serum amylase and lipase levels in patients with AP, and lipase has a higher specificity for the diagnosis of AP than amylase. Elevation of serum amylase and lipase does not affect the severity of AP" (Zhi, 2021).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Amylase

The FDA has approved multiple tests for human serum total amylase as well as for pancreatic amylase.

Lipase

The FDA has approved multiple tests for human serum lipase.

Trypsinogen/Trypsin/TAP

Trypsin immunostaining, trypsinogen-2 dipstick, and TAP serum tests are considered laboratory developed tests (LDT); developed, validated, and performed by individual laboratories.

LDTs are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88).

As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

CRP

The FDA has approved multiple tests for human CRP, including assays for conventional CRP, high sensitivity CRP (hsCRP), and cardiac CRP (cCRP). On September 22, 2005, the FDA issued guidelines concerning the assessment of CRP (FDA, 2005).

Procalcitonin

On April 18, 2017, the FDA approved the Diazyme Procalcitonin PCT Assay, Diazyme Procalcitonin Calibrator Set, and Diazyme Procalcitonin Control Set as substantially equivalent and has received FDA 510K clearance for marketing.

On July 16, 2018, the FDA approved the Atellica IM BRAHMS Procalcitonin (PCT) device which is able to detect and measure procalcitonin (Pct) in human clinical specimens (FDA, 2018).

IL-6/IL-8

IL-6 and IL-8 are ELISA-based tests and are considered laboratory developed tests (LDT); developed, validated, and performed by individual laboratories. IL-6 and IL-8 can also be components of a cytokine panel test, which is also an LDT.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82150	Amylase
83519	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, by radioimmunoassay (e.g., RIA)
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83690	Lipase

84145	Procalcitonin (PCT)
86140	C-reactive protein

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
8/23/22	Modified coverage criteria #1, #2, and #3, and added coverage criteria #5.

Pancreatic Enzyme Testing for Acute Pancreatitis, continued

Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing

Policy #: AHS – G2164	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/28/22 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Parathyroid hormone (PTH), along with calcitriol and fibroblast growth factor 23 (FGF23), regulate calcium and phosphate homeostasis. PTH modulates the serum ionized calcium concentration by stimulating kidney reabsorption of calcium as well as increasing bone resorption within minutes of PTH secretion. Primary hyperparathyroidism presents itself with hypercalcemia and elevated PTH levels and is typically caused by parathyroid adenoma or hyperplasia. Secondary hyperparathyroidism is seen "in patients with kidney failure who have…increased secretion of PTH [and] is related not only to gland hyperplasia and enlargement but also to reduced expression of CaSRs [calcium-sensing receptors] and, perhaps, its downstream signaling elements." (Mannstadt, 2022)

Calcium is an essential metal found in its biologically relevant divalent cation (Ca^{2+}) form in vivo. It is involved in many important biological processes, including cell signaling, signal transduction, and muscle contraction. Only 45% of the plasma calcium is in the ionized form (or 'free' form), which is the physiologically active form, while the rest is bound to albumin or complexed to anions, such as phosphate or citrate (Singh, 2022). Both total calcium and ionized calcium can be tested from a blood sample. Occasionally, calcium concentration is determined from a 24-hour urine sample (AACC, 2014; Fuleihan & Silverberg, 2022).

Phosphorus, a nonmetal, is typically used in its oxidized phosphate polyatomic ionic form (PO_4^{3-}) in vivo and is an important functional group in all classes of biomolecules—carbohydrates, proteins, lipids, and nucleic acids. The cytosol uses a phosphate-based buffer to maintain pH homeostasis. Plasma phosphorus can be in either organic or inorganic form, but the inorganic phosphates are regulated by hormones, primarily PTH. Typically, phosphate/phosphorus testing is performed on a blood sample, but it can also be performed on a urine sample (Alan S, 2022).

Magnesium, like calcium, in vivo is in its divalent cation (Mg^{2+}) form. It is involved in many enzymatic mechanisms as well as structural functions for both proteins and nucleic acids. Magnesium is required

for maintenance of bone health as well as proper nerve conduction, muscle contraction, and energy production. Currently, magnesium is tested from a blood sample or less frequently from a 24-hour urine sample. Due to the large amounts of magnesium that is filtered and the degree of reabsorption and secretion in urine levels, "magnesium levels in the urine do not correlate with either the amount of magnesium ingested or the magnesium status in the body." (Workinger et al., 2018).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Serum intact parathyroid (PTH) testing **MEETS COVERAGE CRITERIA** in the following situations:
    a. To assess possible hyperparathyroidism in individuals with hypercalcemia.
    b. To assess post-operative results of parathyroid surgery.
    c. As part of annual testing of an individual previously diagnosed with hyperparathyroidism.
    d. As part of an assessment of chronic kidney disease (CKD).
    e. As part of an assessment of osteoporosis.
    f. As part of a diagnosis and/or an assessment of cancer or cancer therapy.
2. For individuals suspected of having hypoparathyroidism, pseudohypoparathyroidism, or related disorder, serum intact parathyroid (PTH) testing (See Note 1) **MEETS COVERAGE CRITERIA** in any of the following situations:
    a. In the initial assessment and diagnosis of the disorders listed in Note 1.
    b. To monitor disease and/or therapy.
3. Serum intact parathyroid (PTH) testing to screen for asymptomatic hyperparathyroidism **DOES NOT MEET COVERAGE CRITERIA**.
4. For individuals presenting for a wellness visit or a general exam without abnormal findings, the following tests **DO NOT MEET COVERAGE CRITERIA**:

a. Serum, blood, or fecal magnesium testing.

b. Serum phosphorus or phosphate testing.

c. Urine phosphorus or phosphate testing.

d. Serum total calcium, serum ionized calcium, or urine calcium testing.

e. Serum parathyroid hormone testing.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

5. Testing serum for truncated parathyroid hormone metabolites, including amino-terminal and carboxy-terminal fragments, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

***NOTE 1:** Conditions of hypoparathyroidism, pseudohypoparathyroidism, and related disorders (Mantovani et al., 2018)

1. Hypoparathyroidism
2. Pseudohypoparathyroidism Type 1A (PHP1A)—due to maternal loss of function mutation at the *GNAS* coding sequence
3. Pseudohypoparathyroidism Type 1B (PHP1B)—due to methylation defect at the *GNAS* coding sequence
4. Pseudopseudohypoparathyroidism (PPHP)—due to paternal loss of function mutation at the *GNAS* coding sequence
5. Progressive Osseous Heteroplasia (POH)—due to paternal loss of function mutation at the *GNAS* coding sequence
6. Acrodysostosis (ACRDYS1)—due to mutation in *PRKAR1A*
7. Acrodysostosis (ACRDYS2)—due to mutation in *PDE4D*

III. Scientific Background

Parathyroid hormone (also called parathormone or PTH) is a peptide hormone that is 84 amino acids long when first secreted by the parathyroid gland. It has a biological half-life of approximately 2-4 minutes before being proteolyzed into smaller fragments. These truncated fragments can comprise as much as 95% of the total circulating immunoreactive PTH. PTH is released whenever the serum ionized calcium concentration decreases as detected by the calcium-sensing receptor. Once released, PTH can increase serum calcium concentrations by increasing bone resorption as well as decreasing renal calcium excretion and increasing calcitriol production (Mannstadt, 2022). The bar graph figure below is taken from Valcour et al. (2018), and shows the predominance of the truncated fragments circulating in hemodialysis patients. These truncated PTH peptides can interfere with many serum PTH testing methods (Fuleihan & Juppner, 2022; Valcour et al., 2018).

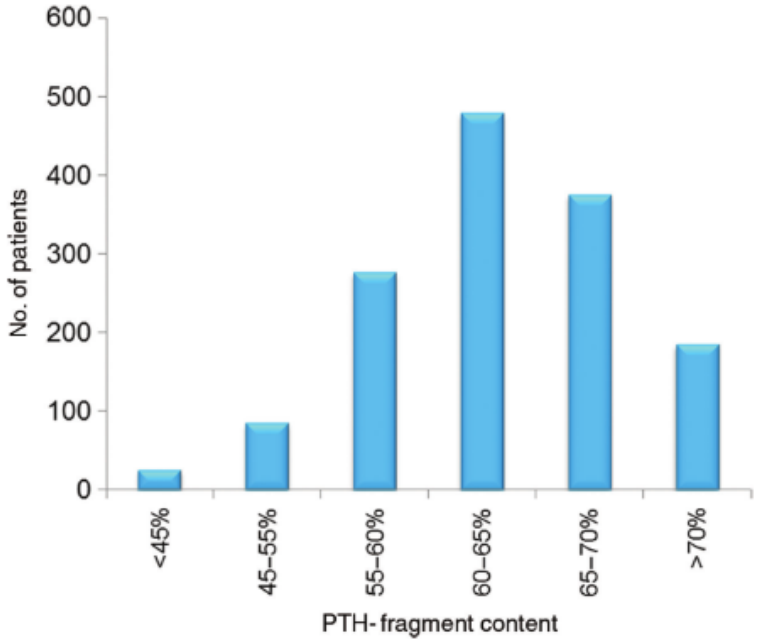

Figure 4: Frequency of fragment content in hemodialysis patients. Representative PTH fragment was assessed in 1533 hemodialysis samples by measuring both PTH(1-84) with the LIAISON method, and iPTH with Method A (high cross-reactivity with inactive fragments [4]). The fragment content was calculated as (iPTH-PTH(1-84))/iPTH and expressed as % of total iPTH.

Both PTH and PTH-related protein analogues may assist in osteoporosis therapy as each play a key role in bone metabolism; it is widely accepted that PTH is an important regulator of calcium homeostasis in the body (Wojda & Donahue, 2018). PTH has been FDA approved as an anabolic treatment for osteoporosis (Wojda & Donahue, 2018). The PTH hormone analog teriparatide is known to stimulate bone remodeling, increase the mineral density in the hip and spine bones, and reduce the risk of fractures in postmenopausal osteoporotic women (Leder, 2017).

Some patients with elevated PTH levels also exhibit vitamin D deficiency, while others do not; however, elevated PTH levels seem to affect both postural stability and muscle function (Bislev et al., 2019). More research needs to be completed in this area.

Hyperparathyroidism is characterized high serum phosphate levels, low serum calcium levels and abnormal PTH levels; this disease is rare and can be managed with active vitamin D and calcium supplements (Marcucci, Della Pepa, & Brandi, 2017). Researchers have noted that treatment with recombinant human parathyroid hormone (rhPTH) may be a good treatment option for patients with hyperthyroidism who cannot maintain normal urinary and serum calcium levels (Marcucci et al., 2017).

The amount of calcium in the bloodstream is monitored by the parathyroid glands. These glands release PTH, which increases blood calcium levels. Magnesium modulates parathyroid hormone secretion; particularly, high magnesium levels increase PTH when the parathyroid glands are exposed to low calcium levels (Rodriguez-Ortiz et al., 2014). Serum calcium may be high due to primary hyperthyroidism and malignancy, or low due to hypothyroidism or renal failure; abnormal serum calcium levels may lead to bone abnormalities or issues in the kidneys, the parathyroid gland, or the gastrointestinal tract (Shaker & Deftos, 2018).

Hypercalcemia is defined as high calcium levels in the blood stream; this may be caused by hyperparathyroidism, drugs, malignancy, or granulomatous disorders (Han, Fry, Sharma, & Han, 2019). Hypercalcemia caused by PTH is the most common cause of primary hyperthyroidism. "Algorithms for diagnosis of PTH related hypercalcaemia require assessment of a 24-h urinary calcium and creatinine excretion to calculate calcium/creatinine clearance ratio and radiological investigations including ultrasound scan and 99mTc-sestamibi-SPECT/CT (Han et al., 2019)."

Serum phosphate homeostasis is principally regulated by the work of PTH and FGF23 via vitamin D. PTH primarily regulates calcium metabolism with secondary effects on phosphate whereas FGF23 is the opposite. Primary hyperparathyroidism (PHPT) often results in hypophosphatemia, but PTH resistance either due to surgical ablation or autoimmune disorders can cause hyperphosphatemia. PTH increases the release of phosphate from bone and the absorption of intestinal phosphate, but it increases the renal excretion of phosphate (Lederer, 2014).

Typically, serum magnesium homeostasis is regulated by the kidneys. However, large increases in PTH increases bone resorption and can also affect the loop of Henle, the location of magnesium reabsorption in the kidneys, to decrease magnesium excretion (Quamme, 1986). Certain types of tumor cells, including esophageal squamous cell carcinomas (ESCC) release a parathyroid hormone-related protein (PTH-rP). A study by Konishi et al. (2018) has demonstrated that PTH and PTH-rP affect magnesium homeostasis in ESCC receiving cisplatin therapy. The researchers found that "intravenous Mg

supplementation therefore conferred protective effects against cisplatin-induced nephrotoxicity in patients with ESCC. Futhermore, increases in PTH or PTH-rP may have influenced the extent of nephrotoxicity (Konishi et al., 2018)." Hernandez-Becerra et al. (2020) recently found that, in rats, a calcium deficiency due to diet results in less magnesium identified in bones, including an apparent lower bone mineral density and a thinner cortical bone and trabecular bone porosity.

Analytical Validity

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a Working Group to research how pre-analytical conditions affected the measurement of PTH in blood samples (Hanon, Sturgeon, & Lamb, 2013). This extensive review covered everything from circadian rhythms and how time of day affected clinical validity to storage conditions and seasonal changes. The research included data from 83 different studies. The authors note that the inclusion of EDTA to the sample will increase the stability to at least 72 hours for plasma samples and to 24 hours for serum samples. PTH concentrations in the summer are lower than in the winter months for patients in the Northern hemisphere, and it is noted that "PTH has a circadian rhythm characterized by a nocturnal acrophase and mid-morning nadir (Hanon et al., 2013)." The data was found to be contradictory concerning the validity of results obtained from frozen samples regardless of whether the sample was stored at -20°C or -80°C. PTH concentrations were also considerably higher in central blood as compared to peripheral blood (median values of 24.3 pmol/L versus 15.3 pmol/L, respectively). It is recommended that "blood samples for PTH measurement should be taken into tubes containing EDTA, ideally between 10:00 [a.m.] and 16:00 [p.m.], and plasma separated within 24 h of venipuncture. Plasma samples should be stored at 4°C and analysed within 72 h of venipuncture. Particular regard must be paid to the venipuncture site when interpreting PTH concentration. Further research is required to clarify the suitability of freezing samples prior to PTH measurement (Hanon et al., 2013)."

The IFCC Working Group on PTH also investigated how to improve PTH testing, especially with regards to the need for common references and standards. "Recent increases in understanding of the complex pathophysiology of CKD, which involves calcium, phosphate and magnesium balance, and is also influenced by vitamin D status and fibroblast growth factor (FGF)-23 production, should facilitate such improvement. Development of evidence-based recommendations about how best to use PTH is limited by considerable method-related variation in results, of up to 5-fold, as well as by lack of clarity about which PTH metabolites these methods recognize. This makes it difficult to compare PTH results from different studies and to develop common reference intervals and/or decision levels for treatment (Sturgeon et al., 2017)." The graph below (taken from (Almond et al., 2012; Sturgeon et al., 2017)) compares the differences between various available PTH assays observed within a single patient specimen.

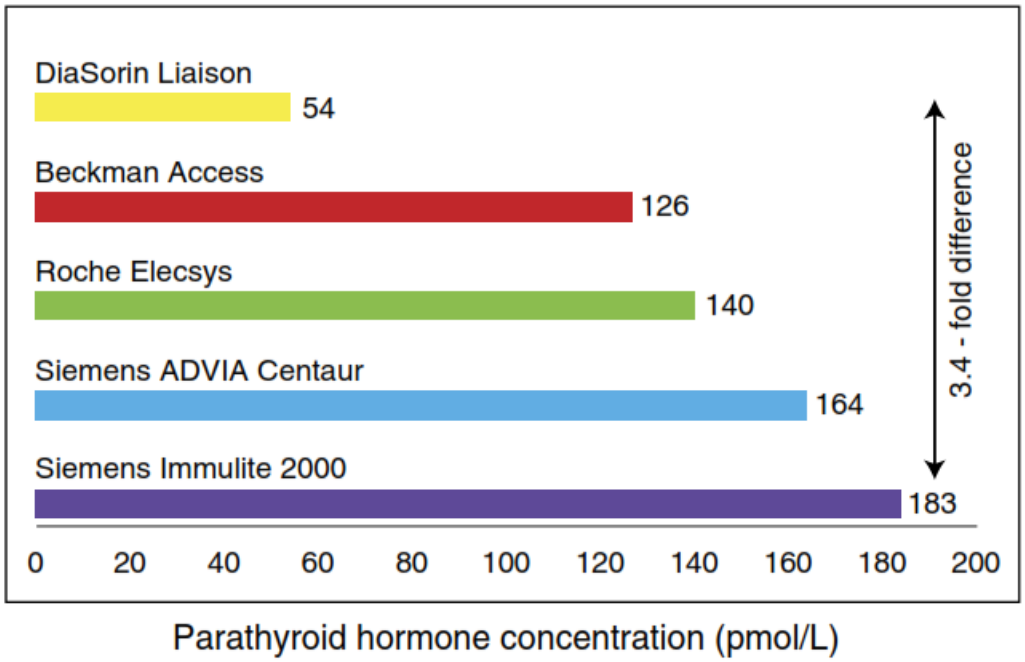

Figure 1 Between-method differences in the concentration of parathyroid hormone (PTH) observed in a typical single patient specimen

The study by Almond et al. (2012) shows that up to 4.2-fold differences can occur between these testing methods, and "these differences were sufficient to have treatment implications for 79% of the patients in the pilot study." The 2017 IFCC study shows that "within-laboratory within-method coefficients of variation (CVs) <10%"; however, "between-laboratory between-method CVs are generally >20%" (Sturgeon et al., 2017).

Bensalah et al. (2018) analyzed the differences in PTH serum measurement between the Roche Cobas e411® (which uses a chemiluminescent sandwich enzyme immunoassay) and the Abbott Architect ci8200® (which uses a chemiluminescent microparticle immunoassay); this study included 252 patients. The two techniques were compared by the Bland-Altman difference diagram. "In conclusion, our study shows a great discrepancy between the results of the PTH assay on the Architect ci8200 versus the Cobas e411", suggesting that currently marketed kits need to be evaluated further (Bensalah et al., 2018).

Clinical Utility and Validity

Since serum PTH testing can be complicated by the presence of proteolytic fragments as well as a brief biological half-life of mere minutes, Valcour et al. (2018) evaluated the efficacy of the LIAISON 1-84 PTH

test, a third-generation serum test, as compared to other intact testing methods. This study was conducted at three different locations throughout the United States. Each test site recruited fifteen patients, and the patients were equally divided into three groups—healthy patients, primary hyperparathyroid patients, and hemodialysis patients. A minimum of nine samples were collected from each patient. Each test's efficacy was also evaluated concerning how the sample was collected (plasma EDTA, unspun plasma EDTA, and serum separator) as well as how storage time at room temperature affected results (up to 72 hours). Two different standards were used—the WHO 95/646 international standard and the synthetic Bachem PTH(1-84) standard. Both the second- and third-generation intact PTH test were consistent with the standards up to 72 hours; however, the "serum is significantly less stable than plasma when samples are stored at room temperature for 72 h regardless of platform, even when separated from the clot by centrifugation within 1 h (Valcour et al., 2018)." The mean percent change from baseline ranged from 96%-107% for the LIAISON 1-84 test except for the serum at 72 h, which had a mean of 82%. Likewise, the second-generation mean percent change from baseline ranged from 95%-108% except for the serum at 72 h, which again was 82%. The authors conclude that the "LIAISON 1-84 PTH assay is accurate and reliably measures the biologically active PTH molecule in plasma or serum stored at room temperature for up [to] 27 and 24 h, respectively (Valcour et al., 2018)."

A study at the Cleveland Clinic of more than 2.7 million patients' electronic medical records was published in 2013 looking at the prevalence of PHPT, both symptomatic and asymptomatic, and the correlation with serum calcium testing. Of the records obtained, 2% had serum calcium levels >10.5 mg/dL, and 1.3% of the total patient population had previously been diagnosed with PHPT. Only 32% of the patients who had not been previously diagnosed with either hypercalcemia, PHPT, or had undergone a parathyroidectomy had recorded PTH values in their medical records. "Patients with calcium of 11.1 – 11.5 mg/dL were most likely to have PHPT (55%). Patients with calcium >12 mg/dL were most likely to have PTH measured. Of hypercalcemic patients, 67% never had PTH obtained, …. It is estimated that 43% of hypercalcemic patients are likely to have PHPT…."; The authors conclude, "it is crucial to evaluate even mild hypercalcemia, because 43% of these patients have PHPT. PHPT is underdiagnosed and undertreated (Press et al., 2013)."

In 1975, Pak and colleagues published results of a urine test they developed to diagnose hypercalciuria (Pak, Kaplan, Bone, Townsend, & Waters, 1975). Since then, 24-hour urinary calcium testing is a common clinical practice, especially in monitoring kidney health, with reference values of <250 mg/24 hours for males and <200 mg/24 hours for females (Mayo, 2018a). A comprehensive study by Curhan, Willett, Speizer, and Stampfer (2001) investigated the 24-hour urine concentrations of calcium, magnesium, and phosphorus along with several other analytes. Calcium and magnesium were quantified by atomic absorption spectroscopy whereas phosphorus was measured using a Cobas centrifugal analyzer. Samples were collected from over 1000 patients who were already taking part in three large-scale ongoing cohort studies—NHS I, NHS II, and HPFS. Neither magnesium nor phosphate was significant in any of the three cohorts between the patients with kidney stones and the controls; however, the urine calcium concentration was significantly elevated ($p \leq 0.01$) in two of the three cohorts. One cohort, though, had 27% of the patients in the control group exhibiting hypercalciuria and only 33% of the experimental group exhibiting hypercalciuria. Conclusions state that "the traditional

definitions of normal 24-hour urine values need to be reassessed, as a substantial proportion of controls would be defined as abnormal… (Curhan et al., 2001)."

Serum magnesium testing can be used in monitoring preeclampsia and hypermagnesemia. The reference values are age-dependent, but levels greater than 9.0 mg/dL can be life-threatening (Mayo, 2018b). The evidence of causation or the use of serum magnesium in predicting preeclampsia have been inconclusive. A study by Kreepala, Kitporntheranunt, Sangwipasnapaporn, Rungsrithananon, and Wattanavaekin (2018) has proposed the use of serum total magnesium and ionized magnesium levels to develop a magnesium-based equation for screening of preeclampsia. This study involved 84 pregnant women including 20 controls. The remaining 64 had been diagnosed with preeclampsia after the 20th week of pregnancy. The authors determined that the serum ionized magnesium levels were "significantly lower in preeclampsia group (23.95 ± 4.7% vs. 26.28 ± 2.3%, p = .04)." The equation that was developed has an "area of ROC for predictive accuracy of the model [of] 0.77 (p < .001) … [The] ROC suggested that the score of 0.27 would be a threshold for screening preeclampsia with 70% sensitivity and 81% specificity." Kreepala et al. (2018) suggest "blood testing on total and ionized magnesium concentrations as well as calculation of ionized magnesium fraction in addition to routine antenatal care for better screening of the disease."

Serum magnesium levels have been identified to play a role in other disorders as well. Low serum magnesium levels have recently been associated with a greater coronary artery disease risk Hamedanian, Badehnoosh, Razavi-Khorasani, Mohammadpour, and Mozaffari-Khosravi (2019); (Rooney et al., 2020). A total of 14446 participants were followed for one year in a large meta-analysis study. The researchers concluded that "low circulating Mg was associated with higher CAD risk than was higher Mg"; however, it was not determined whether magnesium concentration manipulation could assist in the prevention of coronary artery disease (Rooney et al., 2020). Mancuso et al. (2020) conducted a separate study that further validated the association between serum magnesium and CAD. They concluded that Mg2+ could be used to assess subclinical cardiovascular organ damage, including increased carotid artery intima-media thickness and lef ventricular mass index in "hypertensive patients with asymptomatic subclinical vascular atherosclerotic disease and with higher cardiovascular risk." Higher serum Mg2+ concentrations could possibly be protective against progression of CAD as well.

Sri-Ganeshan et al. (2022) conducted a retrospective observational study in a single emergency department, measuring calcium (in 1426 patients), magnesium (in 1296 patients), and phosphate (in 1099 patients). Part of the study involved a clinical tool that analyzed patient electrolyte risk factors, (that is, abnormal calcium, magnesium, and phosphate levels). The "over-testing" of electrolytes in an ER setting is an area of concern, noted the authors. Researchers hypothesized they could use a decision-making tool to determine clinical factors associated with low and high levels of each electrolyte, then only test patients who met the criteria. The authors postulated that "patients without a single risk factor in the tool are unlikely to have clinically significant abnormal Ca, Mg or PO_4 levels and do not require [further] testing." After analyzing results, the authors found very high NPVs for both Ca and Mg, "If Ca and Mg had only been measured in patients with a risk factor for an abnormality, a very small proportion patients (approximately 1%) would not have been identified." However, the authors noted that the use of such a clinical decision-making tool appeared to be less robust when it came to phosphate testing (Sri-Ganeshan et al., 2022).

IV. Guidelines and Recommendations

2016 American Association of Endocrine Surgeons (AAES)

The AAES released guidelines concerning primary hyperparathyroidism (pHPT) in 2016. With respect to laboratory testing, in Recommendation 1-1, these guidelines state, "The biochemical evaluation of suspected pHPT should include serum total calcium, PTH, creatinine, and 25-hydroxyvitamin D levels (strong recommendation; moderate-quality evidence)." The AAES also addresses differentiating between pHPT and suspected "familial hypocalciuric hypercalcemia, which is an autosomal dominant disorder of the renal calcium-sensing receptor that can mimic pHPT." In Recommendation 1-2 (strong recommendation; moderate-quality evidence), "a 24-hour urine measurement of calcium and creatinine should be considered in patients undergoing evaluation for possible pHPT…. Familial hypocalciuric hypercalcemia should be considered in patients with long-standing hypercalcemia, urinary calcium levels less than 10 mg/24 hours, and a calcium to creatinine clearance ratio less than 0.01." The AAES also address the use of intraoperative PTH monitoring (IPM). Recommendation 6-1: "When image-guided focused parathyroidectomy is planned, IPM is suggested to avoid higher operative failure rates (strong recommendation; moderate-quality evidence)." However, a strong recommendation with low-quality evidence to recommendation 6-2 was provided: "Surgeons who use IPM should use a sampling protocol that is reliable in the local environment and should be familiar with the interpretation of PTH decay dynamics." The frequency of testing either calcium or PTH post-operatively is not given, but the AAES mentions these recommendations in several comments concerning the monitoring or measuring calcium and/or PTH levels or determining post-operative hyper-/hypoparathyroidism (Recommendation 14-7, Recommendation 15-1a, Recommendation 15-1b, Recommendation 15-3, Recommendation 15-4, and Recommendation 16-2). It is also stated that the definition of a success versus failure of operation is when levels are compared six months post-operation.

2018 First International Consensus Statement on Pseudohypoparathyroidism and Related Disorders

An international consortium of representatives from across Europe and North America released their first international consensus statement, including extensive guidelines and recommendations, concerning pseudohypoparathyroidism and related disorders in 2018. These disorders have a wide array of phenotypes but are due to impaired cell signaling cascades of G-protein coupled receptors (GPCRs). Pseudohypoparathyroidism can be classified as either type 1A or 1B (PHP1A and PHP1B, respectively), depending on the type of defect in the *GNAS* coding sequence. Pseudopseudohypoparathyroidism (PPHP) and progressive osseous heteroplasia (POH) are caused by a paternal loss of function defect to *GNAS*. Acrodysostosis is classified as either type 1 (ACRDYS1) or type 2 (ACRDYS2) due to mutations in either *PRKAR1A* or *PDE4D*, respectively. PTH resistance can be negligible in infancy but typically increases with age.

In recommendation 1.3 (A+++), the guidelines list the clinical and biochemical major criteria for diagnosing PHP and related disorders, including "PTH resistance, and/or subcutaneous ossifications that can include deeper ossifications, and/or early-onset (before 2 years of age) obesity associated with TSH resistance or with one of the above, and/or AHO [Albright hereditary osteodystrophy] alone" regardless of family history. In recommendation 1.6 (A+++), "The definition of PTH resistance is as follows: [1] The association of hypocalcaemia, hyperphosphataemia and elevated serum levels of PTH in the absence of

vitamin D deficiency and when magnesium levels and renal function are normal. [2] PTH resistance in the context of PHP and related disorders should be suspected when PTH is at, or above, the upper limit of normal, in the presence of normal calcifediol levels and elevated serum levels of phosphorus, even in the absence of overt hypocalcaemia. PTH resistance and consequent changes in serum levels of calcium, phosphorus and PTH can be variable, and repeated testing might be required." In all cases, genetic counseling is recommended.

In recommendation 3.2, the measurement of serum PTH, calcium, phosphorus, and calcifediol are recommended; moreover, "measurement of PTH, calcium and phosphorus should be performed regularly (every 6 months in children and at least yearly in adults) with the exception of patients carrying either a *GNAS* mutation on the paternal allele or a *PDE4D* mutation in whom, apart from diagnosis, routine assessment is not necessary. Monitoring of serum levels of calcium should be more frequent in symptomatic individuals, during acute phases of growth, during acute illness and during pregnancy and breastfeeding…." For patients undergoing vitamin D therapy, they stress as part of recommendation 3.4 (A++) that serum phosphate be monitored. Concerning patients undergoing treatment for PTH resistance, in recommendation 3.5 (A++), the guidelines state that "levels of PTH, calcium and phosphorus should be monitored every 6 months in asymptomatic patients and more frequently when clinically indicated." In recommendation 3.26 (A+), the routine measurement of calcitonin is not recommended. (Mantovani et al., 2018)

2020 European Network on Pseudohypoparathyroidism (EuroPHPnet)

The EuroPHPnet published its "Recommendations for Diagnosis and Treatment of Pseudohypoparathyroidism and Related Disorders: an Updated Practice Tool for Physicians and Patients". In these guidelines, the EuroPHPnet noted that "PTH resistance is the hallmark of PHP [pseudohypoparathyroidism], found in 45-80% of patients", and symptoms of PTH resistance should not be ignored and "screening and follow-up of PTH resistance should include measurement of PTH, 25-OH vitamin D, calcium, and phosphate every 3-6 months in children and at least yearly in adults." However, the frequency of monitoring is also contingent on whether the individual is symptomatic or not, in acute phases of growth, experiencing intercurrent illness, pregnancy, or is breastfeeding. In the case of pregnant women with hypocalcemia and/or hypothyroidism, they "should be monitored following the international guidelines for any pregnancy associated with these disturbances" and their newborns "should be evaluated for the presence of skin ossifications and levels of TSH, calcium, and phosphorous" (Mantovani et al., 2020).

2014 Fourth International Workshop on the Evaluation and Management of Primary Hyperparathyroidism

The Fourth International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism convened in 2014 and published their guidelines as a consensus statement in *The Journal of Bone and Mineral Research*.

For the diagnosis of asymptomatic hypercalcemic PHPT "where biochemical screening is commonly performed, most patients with PHPT come to clinical attention when hypercalcemia is found unexpectedly in the context of an investigation of an unrelated problem or simply upon routine testing. If the PTH level

is also found to be high, or even in the normal range, the most likely diagnosis is asymptomatic hypercalcemic PHPT."

When diagnosing normocalcemic PHPT, "PTH levels may be measured in the evaluation of medical conditions such as osteoporosis, low bone mass, or nephrolithiasis. Normocalcemic PHPT (NPHPT) is characterized by persistently normal albumin-adjusted total and ionized serum calcium levels, accompanied by elevated levels of PTH on at least two consecutive measurements, over a 3-month to 6-month period" (Bilezikian et al., 2022).

The workshop also included a section on genetic testing where they note that testing for mutations in one of 10 genes can facilitate the diagnosis of a syndromic or nonsyndromic form of PHPT, which helps in clinical management and treatment. Specifically, they note that "genetic testing helps to identify family members who may or may not be at risk. Genetic counseling and evaluation, thus, should be considered for patients < 30 years with PHPT, those with multigland disease by history or imaging, those with a family history of hypercalcemia or syndromic diseases such as MEN1, MEN2A, MEN4, or HPT-JT syndrome, and in patients with atypical parathyroid adenoma and parathyroid carcinoma" (Bilezikian et al., 2022).

The 2014 workshop established guidelines for monitoring patients with asymptomatic primary hyperparathyroidism (PHPT) and recommend annual testing of serum calcium. A formula was given to determine corrected calcium concentration, which is recommend rather than using free calcium, since "most centers do not have sufficient capabilities to rely upon an ionized, free calcium concentration":

Corrected [Ca] = [total serum calcium in mg/dL + 0.8*(4.0 - patient's serum albumin in g/dL)]

Recommendations for evaluating asymptomatic PHPT in Table 3 shown below although the guidelines do state that "this evaluation is for PHPT, not to distinguish between PHPT and other causes of hypercalcemia." This table includes calcium (both serum and 24-hour urine testing) and phosphate testing.

Table 3. Recommendations for the Evaluation of Patients With Asymptomatic PHPT

Recommended
Biochemistry panel (calcium, phosphate, alkaline phosphatase activity, BUN, creatinine), 25(OH)D
PTH by second- or third-generation immunoassay
BMD by DXA
Lumbar spine, hip, and distal 1/3 radius
Vertebral spine assessment
X-ray or VFA by DXA
24-h urine for:
Calcium, creatinine, creatinine clearance
Stone risk profile
Abdominal imaging by x-ray, ultrasound, or CT scan
Optional
HRpQCT
TBS by DXA
Bone turnover markers (bone-specific alkaline phosphatase activity, osteocalcin, P1NP [select one]; serum CTX, urinary NTX [select one])
Fractional excretion of calcium on timed urine sample
DNA testing if genetic basis for PHPT is suspected

Abbreviations: BUN, blood urea nitrogen; P1NP, procollagen type 1 N-propeptide; CTX, C-telopeptide cross-linked collagen type I; NTX, N-telopeptide of type I collagen. This evaluation is for PHPT, not to distinguish between PHPT and other causes of hypercalcemia.

In their algorithm for monitoring patients with normocalcemic PHPT, both annual calcium and PTH testing are included; however, there is no mention of the method of calcium testing (i.e. serum versus 24-hour urine testing) or phosphate testing.

National Comprehensive Cancer Network (NCCN)

The NCCN addresses PTH, calcium, phosphate, and magnesium testing in several different guidelines.

Neuroendocrine & Adrenal Tumors (NCCN, 2023g): The NCCN continues to assert that "Primary hyperparathyroidism associated with parathyroid adenomas is the most common manifestation of MEN1 [Multiple endocrine neoplasia, type 1]. Measurement of serum calcium levels is recommended if hyperparathyroidism is suspected. If calcium levels are elevated, parathyroid hormone (PTH) and 25-OH vitamin D levels should be checked." However, in version 2 of the 2020 NCCN guidelines regarding this topic, the NCCN revised their previous guidelines. In the section concerning Multiple Endocrine

Neoplasia, Type 1 (MEN1), the NCCN proposes the use of serum calcium in the diagnosis and clinical evaluation of suspected MEN1, and in the instance that calcium levels are elevated PTH and 25-OH vitamin D be explored. Explicitly, for the evaluation of parathyroid tumors in MEN1, the NCCN states that "Measurement of serum calcium levels is recommended if hyperparathyroidism is suspected. If calcium levels are elevated, parathyroid hormone (PTH) and 25-OH vitamin D levels should be checked." With respect to the surveillance of MEN1-associated parathyroid tumors, "The panel recommends annual calcium and serum PTH levels to screen for parathyroid tumors. If calcium levels rise, 25-OH vitamin D should be measured and imaging with neck ultrasound and/or parathyroid sestamibi with SPECT scan (SPECT-CT preferred) or 4D-CT should be performed." Similarly, for the evaluation of patients with Multiple Endocrine Neoplasia Type 2 (MEN2), "serum calcium levels should be measured. If it is found to be elevated, PTH and 25-OH vitamin D levels should be measured. A neck ultrasound, sestamibi scan with SPECT, or 4D-CT scan can also be performed as appropriate."

Acute Lymphoblastic Leukemia (ALL) (NCCN, 2022): As part of the initial workup for ALL patients, they recommend "a tumor lysis syndrome (TLS) panel (including measurements for serum lactate dehydrogenase [LDH], uric acid, potassium, phosphates, and calcium)." In the section concerning the supportive care of ALL in steroid management, they guidelines state to "obtain vitamin D and calcium status and replete as needed" to monitor possible osteonecrosis/avascular necrosis associated as a potential long-term side effect of corticosteroids. Likewise, the NCCN later stated, "To monitor patients for risks of developing symptomatic osteonecrosis, routine measurements for vitamin D and calcium levels should be obtained and periodic radiographic evaluation (using plain films or MRI [magnetic resonance imaging]) should be considered (NCCN, 2022a)."

Systemic Light Chain Amyloidosis (NCCN, 2023j): As part of the initial diagnostic workup, in the section titled "Laboratory evaluation (directed toward commonly affected organ systems)," the NCCN recommends testing "serum BUN/creatinine, electrolytes, albumin, calcium, serum uric acid, serum LDH, and beta-2 microglobulin (NCCN, 2020k)."

Bone Cancer (NCCN, 2023b): **:** In the section concerning the workup of Giant Cell Tumor of Bone (GCTB), a rare benign tumor, the guidelines state that "brown tumor of hyperparathyroidism should be considered as a differential diagnosis; routine evaluation of serum calcium, phosphate, and parathyroid hormone levels can help exclude this diagnosis." Moreover, prior to treatment of bone lesions, it is recommended that: "Laboratory studies, such as complete blood count (CBC), comprehensive metabolic panel (CMP) with calcium to assess for hypercalcemia, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) should be done prior to initiation of treatment."

Breast Cancer (NCCN, 2021b): In general, in monitoring metastatic disease, "laboratory tests such as alkaline phosphatase, liver function, blood counts, and calcium…" are to be included to help aid the clinician in determining "the effectiveness of treatment and the acceptability of toxicity."

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) (NCCN, 2023C): Small-molecule inhibitors, such as Venetoclax, are possible therapies for CLL/SLL. Tumor Lysis Syndrome (TLS) is a possible side effect of such treatment. In the section on supportive care for CLL/SLL, they note that "patients with bulky lymph nodes, progressive disease after small-molecular inhibitor therapy, and

receiving chemotherapy, venetoclax, lenalidomide, obintuzumab are considered to be at high-risk for TLS." NCCN further states that laboratory hallmarks of TLS include high potassium, uric acid, phosphorous, lactate dehydrogenase, and low calcium. In Venetoclax therapy, particularly, they state to "evaluate blood chemistries (potassium, uric acid, phosphorus, calcium, and creatinine); review in real time." The table below (adapted from the guideline) depicts the blood chemistry monitoring as recommended:

Blood Chemistry Monitoring (potassium uric acid, phosphorus, calcium and creatinine)	
Low Tumor Burden	
Outpatient setting	Pre-dose, 6-8 hours, 24 hours at first dose of 20mg and 50 mg Pre-dose at subsequent ramp-up doses
Medium Tumor Burden	
Outpatient setting	Pre-dose, 6-8 hours, 24 hours at first dose of 20mg and 50mg Pre-dose at subsequent ramp-up doses Consider hospitalization for patients with CrCl <80 mL/min at first dose of 20 mg and 50 mg
High Tumor Burden	
In hospital setting	At first dose of 20 and 50 mg Pre-dose 4 hrs 8 hrs 12 hrs 24 hrs
Outpatient setting (for subsequent ramp-up doses)	Pre-dose 6-8 hrs 24 hrs

Esophageal and Esophagogastric Junction Cancers (NCCN, 2023d): In the section on principles of survivorship under *Management of Long-Term Sequelae of Disease or Treatment*, they say to "consider monitoring vitamin B, folic acid, vitamin D, and calcium levels." Moreover, following esophagectomy, long-term calcium deficiency is common along with deficiencies in vitamin B_{12}, folic acid, and vitamin D (NCCN, 2020c)

Kidney Cancer (NCCN, 2023e): The NCCN uses serum calcium levels as a predictor of short survival used to select patients for temsirolimus, as well as a prognostic factor [i.e. "calcium > upper limit of normal (Normal: 8.5-10.2 mg/dL)"]. The guidelines do not state how frequently serum calcium should be tested or if it is solely for use at diagnosis. However, the guidelines recommend that laboratory evaluation for

patients with renal cell carcinoma typically present with a suspicious mass involving the kidney may include a metabolic panel consisting of "corrected calcium, serum creatinine, liver function studies, and urinalysis" (NCCN, 2020d).

Multiple Myeloma (NCCN, 2023f): In the initial diagnostic workup for multiple myeloma, the NCCN recommends testing "serum BUN/creatinine, electrolytes, albumin, calcium, serum uric acid, serum LDH, and beta-2 microglobulin (NCCN, 2020f)." As follow-up to the clinical presentation of either "solitary plasmocytoma" (with minimal marrow involvement or less) or "smoldering (asymptomatic)" myeloma, again "corrected calcium" is listed as one of the recommended blood tests. Calcium is also recommended following treatment of active myeloma, and an elevated calcium concentration is listed as one of the "direct indicators of increasing disease and/or end organ dysfunction" since "excess bone resorption from bone disease can lead to excessive release of calcium into the blood, contributing to hypercalcemia" (NCCN, 2020e, 2020f).

Occult Primary (Cancer of Unknown Primary [CUP]) (NCCN, 2023h): "Routine laboratory tests (ie, complete blood count [CBC], electrolytes, liver function tests, creatinine, calcium…)are also recommended." (NCCN, 2020i)

Prostate Cancer (NCCN, 2023i): In the section concerning the treatment with denosumab, the guidelines state that "hypocalcemia is seen twice as often with denosumab than zoledronic acid and all patients on denosumab should be treated with vitamin D and calcium with periodic monitoring of serum calcium levels (NCCN, 2018c, 2020j)." In the section concerning patients with castration resistant prostate cancer (CRPC), the NCCN states, "hypocalcemia should be corrected before starting denosumab, and serum calcium monitoring is required for denosumab and recommended for zoledronic acid, with repletion as needed." In treatment of CRPC with abiraterone acetate, "monitoring of liver function, potassium and phosphate levels, and blood pressure readings on a monthly basis is warranted during abiraterone therapy." Men with CRPC are at a higher risk for severe hypocalcemia and hypophosphatemia due to use of denosumab (NCCN, 2021b)

T-Cell Lymphomas (NCCN, 2023k): For adult T-Cell Leukemia/Lymphoma (ATLL), the NCCN states, "the initial workup for ATLL should include a complete history and physical examination…a CBC with differential and complete metabolic panel (serum electrolyte levels, calcium, creatinine, and blood urea nitrogen) and measurement of serum LDH levels ." Under the supportive care section for T-Cell lymphomas, the NCCN recommends monitoring for tumor lysis syndrome (TLS), which include measuring serum phosphorous and calcium levels since "laboratory TLS is defined as a 25% increase in the levels of serum uric acid, potassium, or phosphorus or a 25% decrease in calcium levels" (NCCN, 2020l).

Thyroid Carcinoma (NCCN, 2023l): In the algorithm for thyroid carcinoma-medullary carcinoma, both serum calcium and PTH are recommended as additional workup for patients who have MEN2A/Familial medullary thyroid carcinoma (codon 609, 611, 618, 620, 630, 634, 768, 790, 791, 804, or 891 *RET* mutations). Serum calcium testing is among the testing and procedures recommended upon diagnosis of medullary thyroid carcinoma (NCCN, 2020m).

2012, 2017 Kidney Disease Improving Global Outcomes (KDIGO)

KDIGO released their *Clinical practice guideline for the Evaluation and Management of Chronic Kidney Disease* (CKD) in 2012 and then their *Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)* in 2017. In the 2012 guidelines (KDIGO, 2013), in recommendation 3.3.1 (1C), they state, "We recommend measuring serum levels of calcium, phosphate, PTH, and alkaline phosphatase activity at least once in adults with GFR <45 ml/min/1.73 m^2 (GFR categories G3b-G5) in order to determine baseline values and inform prediction equations if used." In recommendation 3.3.4 (2C recommendation strength), for people in GFR categories G3b-G5 they "suggest that people with levels of intact PTH above the upper normal limit of the assay are first evaluated for hyperphosphatemia, hypocalcemia, and vitamin D deficiency." With regards to serum phosphate levels, they recommend that they are maintained "in the normal range according to local laboratory reference values" (recommendation 3.3.3; 2C). The guidelines, however, do not state a recommendation with respect to the frequency of testing past initial baseline and do not address magnesium testing other than to list renal magnesium wasting as a criterion for CKD.

The 2017 guidelines (KDIGO, 2017) in recommendation 3.1.1 state: "We recommend monitoring serum levels of calcium, phosphate, PTH, and alkaline phosphatase activity beginning in CKD G3a (*1C*). In children, we suggest such monitoring beginning in CKD G2 (*2D*)." Recommendation 3.1.2 (*Not graded*) addresses the frequency of such testing and says, "To base the frequency … on the presence and magnitude of abnormalities, and the rate of progression of CKD." The table below lists the "reasonable monitoring intervals":

CKD Stage	Test	Reasonable Monitoring Interval
G3a-G3b	Serum Calcium	Every 6-12 months
G3a-G3b	Serum Phosphate	Every 6-12 months
G3a-G3b	PTH	"Based on baseline level and CKD progression"
G4	Serum Calcium	Every 3-6 months
G4	Serum Phosphate	Every 3-6 months
G4	PTH	Every 6-12 months
G5	Serum Calcium	Every 1-3 months
G5	Serum Phosphate	Every 1-3 months
G5	PTH	Every 3-6 months

CKD Stage	Test	Reasonable Monitoring Interval
G4-G5D	Alkaline Phosphatase Activity	Every 12 months, or more frequently in the presence of elevated PTH

Recommendation 3.2.3 (*2B*) suggests measuring either PTH or bone-specific alkaline phosphatase to assess bone disease. For patients with CKD G3a-G5D, their treatment "should be based on serial assessments of phosphate, calcium, and PTH levels, considered together" (Recommendation 4.1.1 *Not Graded*). Recommendation 4.2.1 (*2C*) states: "In patients with CKD G3a-G5 not on dialysis, the optimal PTH level is not known. However, we suggest that patients with levels of intact PTH progressively rising or persistently above the upper normal limit for the assay be evaluated for modifiable factors, including hyperphosphatemia, hypocalcemia, high phosphate intake, and vitamin D deficiency." Recommendation 5.2 (*Not Graded*) addressed the frequency of testing post-kidney transplant. The table below contains the information regarding the reasonable monitoring intervals:

CKD Stage	Test	Reasonable Monitoring Interval
G1T-G3bT	Serum Calcium	Every 6-12 months
G1T-G3bT	Serum Phosphate	Every 6-12 months
G1T-G3bT	PTH	Once, with subsequent intervals depending on baseline level and CKD progression
G4T	Serum Calcium	Every 3-6 months
G4T	Serum Phosphate	Every 3-6 months
G4T	PTH	Every 6-12 months
G5T	Serum Calcium	Every 1-3 months
G5T	Serum Phosphate	Every 1-3 months
G5T	PTH	Every 3-6 months
G3aT-G5T	Alkaline Phosphatase Activity	Annually, or more frequently in the presence of elevated PTH

Within recommendation 5.6 (*2C*), KDIGO recommends "treatment choices be influenced by the presence of CKD-MBD, as indicated by abnormal levels of calcium, phosphate, PTH, alkaline phosphatases, and 25(OH)D (KDIGO, 2017)."

American Urological Association (AUA)

In 2013, the AUA published Follow-up for Clinically Localized Renal Neoplasms. In recommendation 2, as an Expert Opinion, the AUA states, "Patients undergoing follow-up for treated or observed renal masses should undergo basic laboratory testing to include blood urea nitrogen (BUN)/creatinine, urine analysis (UA) and estimated glomerular filtration rate (eGFR). Other laboratory evaluations, including complete blood count (CBC), lactate dehydrogenase (LDH), liver function tests (LFTs), alkaline phosphatase (ALP) and calcium level, may be used at the discretion of the clinician."

The AUA published their guidelines titled *Medical Management of Kidney Stones* in 2014. These guidelines were reviewed, and validity was confirmed in 2019 (Pearle et al., 2019). In recommendation 2, the AUA recommends that "clinicians should obtain serum intact parathyroid hormone (PTH) level as part of the screening evaluation if primary hyperparathyroidism is suspected." Also recommend (Recommendations 6 & 7) is that "metabolic testing should consist of one or two 24-hour urine collections obtained on a random diet and analyzed at minimum for total volume, pH, calcium, oxalate, uric acid, citrate, sodium, potassium and creatinine" but that "clinicians should not routinely perform 'fast and calcium load' testing to distinguish among types of hypercalciuria (M. Pearle et al., 2019)."

2014-2021 National Institute for Health and Care Excellence (NICE)

NICE, like the NCCN, addresses PTH, calcium, phosphate, and magnesium testing in several different guidelines.

2014 Bipolar disorder: assessment and management (NCCMH, 2020): In recommendation 1.2.12, they recommend annual calcium screening for anyone on a long-term lithium therapy regimen; however, in recommendation 1.10.21, they recommend testing "for urea and electrolytes including calcium…every six months, and more often if there is evidence of impaired renal or thyroid function, raised calcium levels or an increase in mood symptoms that might be related to impaired thyroid function." In recommendation 1.10.14, when a patient begins a lithium regimen, a clinician should test "for urea and electrolytes including calcium, estimated glomerular filtration rate (eGFR), thyroid function and a full blood count."

2014 Multiple sclerosis in adults: management (NICE, 2019): In recommendation 1.1.4, they recommend calcium testing along with full blood count, inflammatory markers, liver and renal function tests, glucose, thyroid function tests, vitamin B_{12}, and HIV [human immunodeficiency virus] serology testing "before referring a person suspected of having MS to a neurologist" to "exclude alternative diagnoses."

2015 Suspected cancer: recognition and referral (NICE, 2021b): In the section concerning myeloma, in recommendation 1.10.4, they state, "offer a full blood count, blood tests for calcium and plasma viscosity or erythrocyte sedimentation rate to assess for myeloma in people aged 60 and over with persistent bone pain, particularly back pain, or unexplained fracture."

2019 Clinical practice guideline: undernutrition in chronic kidney disease (Wright et al., 2019): These guidelines include a section regarding the nutritional status of an individual with chronic kidney disease. The NICE states that "Assessment of nutritional status should therefore be considered when patients begin education for kidney replacement treatment as part of their overall care as well as for potential

intervention regarding salt, potassium, phosphate and protein / energy intake assessments (Wright et al., 2019)." Specific assessment methods are not mentioned.

2021 Chronic kidney disease: assessment and management (NICE, 2021a): In recommendation 1.11.9, within the section concerning the use of phosphate binders for children and young people, they state to "offer children and young people with CKD stage 4 or 5 and hyperphosphataemia a calcium-based phosphate binder to control serum phosphate levels." In the continuation via recommendation 1.11.10, they also state, "if serum calcium increases towards, or above, the age-adjusted upper normal limit: ●investigate possible causes other than the phosphate binder ●consider reducing the dose of the calcium-based phosphate binder and adding sevelamer carbonate or switching to sevelamer carbonate alone [2021]." When discussing phosphate binders for adults, they state in their recommendation 1.11.12 for the first phosphate binder, "offer adults with CKD stage 4 or 5 and hyperphosphataemia calcium acetate to control serum phosphate levels [2021]." If calcium acetate is not indicated, "for example, because of hypercalcaemia or low serum parathyroid hormone levels," or not tolerated, recommendation 1.11.13 states to offer sevelamer carbonate. Recommendations 1.11.14 and 1.11.15 continue by offering sucroferric oxyhydroxide, if an adult is on dialysis and a calcium-based phosphate binder is not needed; calcium carbonate, "if a calcium-based phosphate binder is needed"; and lanthanum carbonate "for adults with CKD stage 4 or 5 if other phosphate binders cannot be used." In the 2021 update, they also state in recommendation 1.11.18, "at every routine clinical review, assess the person's serum phosphate control, taking into account: ●diet ●whether they are taking the phosphate binders as prescribed ●other relevant factors, such as vitamin D levels, serum parathyroid hormone levels, alkaline phosphatase, serum calcium, medications that might affect serum phosphate, or dialysis [2021]". These guidelines mention serum phosphate, serum calcium, and PTH; however, they do not state when these tests should be performed or the frequency of testing.

In recommendation 1.12.1, they do not recommend to "routinely measure calcium, phosphate, parathyroid hormone (PTH) and vitamin D levels in people with a GFR of 30 ml/min/1.73 m^2 or more (GFR category G1, G2, or G3)." Then, in the following recommendation, they do recommend measuring serum calcium, PTH, and phosphate for patients in GFR categories G4 or G5. "Determine the subsequent frequency of testing by the measured values and the clinical circumstances. If doubt exists, seek specialist opinion." They recommend in 1.12.7 to "monitor serum calcium and phosphate concentrations in people receiving alfacalcidol or calcitriol supplements."

2021 American Society of Clinical Oncology (ASCO)/Cancer Care Ontario (CCO)

The CCO and ASCO convened a working group in 2017 concerning the use of bisphosphonates in breast cancer and published their recommendations in the *Journal of Clinical Oncology*. They clearly state that "patients should have serum calcium measured prior to starting treatment. Patients receiving intravenous bisphosphonates (zoledronic acid) should be monitored for renal function prior to starting this treatment, and for serum calcium and increase in serum creatinine throughout the treatment period."

A 2021 update from the CCO and ASCO group reaffirmed the statement above (Eisen et al., 2022).

American Association of Clinical Endocrinologists (AACE)/American College of Endocrinology (ACE)

In 2016, the AACE/ACE posted guidelines concerning osteoporosis in post-menopausal women recommending PTH, phosphate, and 24-hour urine calcium testing in evaluating osteoporosis. The guidelines note that "the 24-hour urine calcium collection must occur after the patient is vitamin D replete and has been on a reasonable calcium intake (1,000-1,200 mg/day) for at least 2 weeks (Camacho et al., 2020)."

In the 2017 guidelines for the management of dyslipidemia prevention of cardiovascular disease, the AACE/ACE highlighted the use of coronary artery calcium scores in the detection of cardiovascular risk, stating that coronary artery calcium scoring "is recognized by the AHA as a surrogate marker for coronary heart disease" (Jellinger et al., 2017)

2014 Society of Obstetricians and Gynaecologists of Canada (SOGC)

The 2014 SOGC guidelines concerning hypertensive disorders during pregnancy recommend using magnesium supplements for pregnant women; however, the SOGC clearly states in recommendation #120 that "routine monitoring of serum magnesium levels is not recommended (Magee et al., 2014)."

2022 American Heart Association (AHA) /American College of Cardiology (ACC) /Heart Failure Society of America (HSFA) Guideline for the Management of Heart Failure

The 2022 guideline concerning heart failure mentions both magnesium and calcium testing for patients with heart failure (HF), "Laboratory evaluation with complete blood count, urinalysis, serum electrolytes (including sodium, potassium, calcium, and magnesium), blood urea nitrogen, serum creatinine, glucose, fasting lipid profile, liver function tests, iron studies (serum iron, ferritin, transferrin saturation), and thyroid-stimulating hormone level and electrocardiography is part of the standard diagnostic evaluation of a patient with HF" (Heidenreich et al., 2022).

2016 American Academy of Pediatrics (AAP)

The AAP in 2016 issued guidelines concerning Brief Resolved Unexplained Events (BRUE) in infants. "The term BRUE is defined as an event occurring in an infant younger than 1 year when the observer reports a sudden, brief, and now resolved episode of ≥1 of the following: (1) cyanosis or pallor; (2) absent, decreased, or irregular breathing; (3) marked change in tone (hyper- or hypotonia); and (4) altered level of responsiveness." For infants between 60 days and <1 year in age, in recommendation 6B under IEM (inborn error of metabolism), the AAP states that "clinicians should not obtain a measurement of serum sodium, potassium, chloride, blood urea nitrogen, creatinine, calcium, or ammonia to detect an IEM on infants presenting with a lower-risk BRUE (Grade C, Moderate Recommendation) (Tieder et al., 2016)."

2013 American Association of Clinical Endocrinologists (AACE)/American College of Endocrinology (ACE)/The Obesity Society (TOS)

The joint task force between AACE, ACE, and TOS issued *Clinical Practice Guidelines for Healthy Eating for the Prevention and Treatment of Metabolic and Endocrine Diseases in Adults* in 2013. With regards to CKD in recommendation R29, they state, "If the intact parathyroid hormone (PTH) level remains

elevated above treatment goal despite a serum 25(OH)D level higher than 30 ng/mL, treatment with an active form of vitamin D is indicated (Grade A, BEL 1)." As part of recommendation R32, they state, "A 24-hour urine calcium collection should be measured in patients with osteoporosis or patients at risk for bone loss in order to check calcium adequacy and test for hypercalciuria or malabsorption (Grade B, BEL 2)." Furthermore, "during vitamin D therapy, serum calcium and phosphorus levels need to be monitored closely to prevent hypercalcemia and hyperphosphatemia, aiming for calcium and phosphorus levels of <10.2 mg/dL and <4.6 mg/dL, respectively."

2013, 2019 AACE/TOS/ASMBS (American Society for Metabolic & Bariatric Surgery)/OMA (Obesity Medical Association)/ASA (American Society of Anesthesiologists)

Also, in 2013, the AACE/TOS/ASMBS/OMA/ASA issued guidelines concerning perioperative, nonsurgical support for the bariatric surgery patient. Within recommendation R48, they state, "Bisphosphonates may be considered in bariatric surgery patients with osteoporosis only after appropriate therapy for calcium and vitamin D insufficiency…. Evaluation should include serum parathyroid hormone (PTH), total calcium, phosphorus, 25-hydroxyvitamin D, and 24-hour urine calcium levels (Grade C; BEL 3)."

The updated guidelines for the perioperative nutrition, metabolic, and nonsurgical support of patients undergoing bariatric procedures were published by the ACE, TOS, ASMBS as well as the Obesity Medicine Association, and the American Society of Anesthesiologists Boards of Directors. The guidelines give the following recommendations:

- "Patients who become pregnant following bariatric procedure should have nutritional surveillance and laboratory screening for nutrient deficiencies every trimester, including iron, folate, vitamin B12, vitamin D, and calcium, and if after a malabsorptive procedure, fat-soluble vitamins, zinc, and copper (Grade D)
- Evaluation of patients for bone loss after bariatric procedures may include serum parathyroid hormone, total calcium, phosphorus, 25-hydroxyvitamin D, and 24-hour urine calcium levels (Grade C; BEL 3) (Mechanick et al., 2019)."

2013 American Gastroenterological Association (AGA)

The 2013 AGA guidelines concerning constipation states that "although metabolic tests (thyroid-stimulating hormone, serum glucose, creatinine, and calcium) are often performed, their diagnostic utility and cost-effectiveness have not been rigorously evaluated and are probably low." Under the section *What Tests Should Be Performed to Assess for Medical Causes of Constipation?*, they state, "In the absence of other symptoms and signs, only a complete blood cell count is necessary (strong recommendation, low-quality evidence). Unless other clinical features warrant otherwise, metabolic tests (glucose, calcium, sensitive thyroid-stimulating hormone) are not recommended for chronic constipation (strong recommendation, moderate-quality evidence)."

American Thyroid Association (ATA)

The ATA has published guidelines for the diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. The ATA has stated that after a thyroidectomy, "serum calcium with or without intact parathyroid hormone (iPTH) levels can be measured,"; further, after a thyroidectomy for TMNG (toxic multinodular goiter), "serum calcium with or without iPTH levels should be measured" (Ross et al.,

2016). When preparing patients with GD (Graves' disease) for a thyroidectomy, the ATA recommends that "Calcium and 25-hydroxy vitamin D should be assessed preoperatively and repleted if necessary (Ross et al., 2016)."

The ATA also published a statement regarding postoperative hypoparathyroidism. In it, they recommend to "Either treat at-risk patients empirically with calcium, or measure calcium and/or PTH in the immediate postoperative period and treat according to evidence-based protocols." (Orloff et al., 2018)

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82310	Calcium; total
82330	Calcium; ionized
82340	Calcium; urine quantitative, timed specimen
83735	Magnesium
83970	Parathormone (parathyroid hormone)
84100	Phosphorus inorganic (phosphate)
84105	Phosphorus inorganic (phosphate); urine

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
4/28/22	Modified coverage criteria #1a to now read as follows: "To assess for possible hyperparathyroidism in patients with hypercalcemia; OR"

Pathogen Panel Testing

Policy #: AHS – G2149	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/27/22, 8/22/22, 3/20/23, 4/3/24 (see Section VIII)

a.

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Infectious diseases can be caused by a wide range of pathogens. Conventional diagnostic methods like culture, microscopy with or without stains and immunofluorescence, and immunoassay often lack sensitivity and specificity and have long turnaround times. Panels for pathogens using multiplex amplified probe techniques and multiplex reverse transcription can detect and identify multiple pathogens in one test using a single sample (Palavecino, 2015).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. In the outpatient setting, multiplex PCR-based panel testing of (up to **5** gastrointestinal pathogens [GIPs]) **MEETS COVERAGE CRITERIA** for individuals with any of the following conditions:

   a. Community-acquired diarrhea of ≥ 7 days duration.

   b. Diarrhea with signs or risk factors for severe disease (fever, bloody diarrhea, dysentery, dehydration, severe abdominal pain, hospitalization and/or immunocompromised state).

2. In the outpatient setting, multiplex PCR-based panel testing (up to **11** GIPs) **MEETS COVERAGE CRITERIA** for immunosuppressed or HIV positive patients who have any of the following conditions:

a. Community-acquired diarrhea of ≥ 7 days duration.

b. Diarrhea with signs or risk factors for severe disease (fever, bloody diarrhea, dysentery, dehydration, severe abdominal pain, hospitalization and/or immunocompromised state).

3. In the outpatient setting, multiplex PCR-based panel testing (up to **5** respiratory pathogens) **MEETS COVERAGE CRITERIA** for patients who are displaying signs and symptoms of a respiratory tract infection, including at least one of the following:

   a. A temperature ≥ 102°F.

   b. Pronounced dyspnea.

   c. Tachypnea.

   d. Tachycardia.

4. In the outpatient setting, qualitative PCR testing for corneal ulcers where non-bacterial etiologies (e.g., fungal, acanthamoeaba, herpes simplex virus (HSV)/herpes zoster virus (HZV)) are suspected **MEETS COVERAGE CRITERIA**.

5. In the outpatient setting, multiplex PCR-based panel testing of **12 or MORE** GIPs **DOES NOT MEET COVERAGE CRITERIA.**

6. In the outpatient setting, multiplex PCR-based panel testing of **6 or MORE** respiratory pathogens **DOES NOT MEET COVERAGE CRITERIA**.

7. In the outpatient setting, multiplex PCR-based panel testing of pathogens in cerebrospinal fluid (CSF) **DOES NOT MEET COVERAGE CRITERIA.**

8. In the outpatient setting, molecular detection-based panel testing of pathogens in the blood **DOES NOT MEET COVERAGE CRITERIA.**

9. In the outpatient setting, molecular detection-based panel testing of pathogens for urinary tract infection, sinusitis, and prostatitis, or other conditions not listed above are not medically necessary, as they are not superior to standard of care testing and **DO NOT MEET COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

10. In the outpatient setting, molecular detection-based panel testing of urine pathogens for the diagnosis of urinary tract infections (e.g., GENETWORx Molecular PCR UTI Test) **DOES NOT MEET COVERAGE CRITERIA**.

11. In the outpatient setting, molecular-based panel testing to screen for or to diagnose wound infections (e.g., GENETWORx PCR Wound Testing) **DOES NOT MEET COVERAGE CRITERIA**.

12. Molecular-based panel testing for general screening of microorganisms (e.g., MicroGenDX qPCR+ NGS) **DOES NOT MEET COVERAGE CRITERIA.**

III. Scientific Background

There has been a move in recent years towards employing molecular tests that use multiplex polymerase chain reaction (PCR) to simultaneously detect multiple pathogens associated with an infectious disease rather than one organism. These tests are usually offered as a panel for a particular infectious condition, such as sepsis and blood stream infections, central nervous system infections (for example, meningitis and encephalitis), respiratory tract infections, urinary tract infections or gastrointestinal infections. These assays are often more sensitive than conventional culture-based or antigen detection. The high diagnostic yield is particularly important when clinical samples are difficult to collect or are limited in volume (e.g., CSF). Multiplex PCR assays are also particularly beneficial when different pathogens can cause the same clinical presentation, thus making it difficult to narrow down the causative pathogen. Access to comprehensive and rapid diagnostic results may lead to more effective early treatment and infection-control measures. Disadvantages of multiplex PCR assays include high cost of testing and potential false negative results due to preferential amplification of one target over another (Palavecino, 2015).

The Centers for Medicare and Medicaid Services (CMS, 2021) report that the top target pathogens causing infections include *Salmonella*, *Campylobacter*, *Shigella*, *Cryptosporidium*, Shiga toxin producing *E. coli* non-O157 and Shiga toxin producing *E. coli* O157; these pathogens "represent the top 90-95% of foodborne infections [incidence of infection per 100,000 population]" (CMS, 2022)

Proprietary Testing

Gastrointestinal Pathogen Panel

Approximately 1.7 billion cases of childhood diarrheal disease occur worldwide every year, resulting in about 525,000 deaths in children younger than five years of age annually (WHO, 2017). The Centers for Disease Control and Prevention (CDC) has estimated that nearly 48 million cases of acute diarrheal infection occur annually in the United States, at an estimated cost upwards of $150 million (Scallan et al., 2011). Approximately 31 major pathogens acquired in the United States caused an estimated 9.4 million episodes of diarrheal illness, 55,961 hospitalizations, and 1,351 deaths each year. Additionally, unspecified agents caused approximately 38 million episodes of foodborne illnesses and resulted in 71,878 hospitalizations and 1,686 deaths. Diarrhea can be classified as acute (lasting less than 14 days), persistent (14 and 30 days), and chronic (lasting for greater than a month) (Riddle et al., 2016). Further, healthcare and antibiotic associated diarrhea are mainly caused by toxin-producing *Clostridium difficile* causing more than 300,000 cases annually (CMS, 2022).

Acute infectious gastroenteritis is generally associated with other clinical features like fever, nausea, vomiting, severe abdominal pain and cramps, flatulence, bloody stools, tenesmus, and fecal urgency. A wide spectrum of enteric pathogens can cause infectious gastroenteritis, including bacteria such as

Campylobacter, *Clostridium difficile*, *Salmonella*, *Shigella*, *Vibrio* and *Yersinia*; viruses, such as Norovirus, Rotavirus, Astrovirus and Adenovirus; and parasites, such as *Giardia*, *Entamoeba histolytica* and *Cryptosporidium* (Riddle et al., 2016).

Stool culture is the primary diagnostic tool for a suspected bacterial infection, but it is time-consuming and labor intensive. Stool samples are collected and analyzed for various bacteria present in the lower digestive tract via cell culture; these bacteria may be normal or pathogenic (Humphries & Linscott, 2015). By identifying the type of bacteria present in a stool sample, a physician will be able to determine if the bacteria are causing gastrointestinal problems in an individual. However, stool culture has a low positive yield. Similarly, methods like electron microscopic examination and immunoassay that are used to diagnose viruses are labor intensive and need significant expertise (Zhang, Morrison, & Tang, 2015). Multiplex PCR-based assays have shown superior sensitivity to conventional methods for detection of enteric pathogens and are increasingly used in the diagnosis of infectious gastroenteritis. These assays have significantly improved workflow and diagnostic output in the diagnosis of gastrointestinal infections (Zhang et al., 2015). Several FDA-approved multiplex PCR assays are now commercially available. Some assays can detect only bacterial pathogens in stool, whereas others can detect bacterial, viral, and parasitic pathogens. The Strong-LAMP assay is a technique which uses PCR to detect Strongyloides in stool and urine samples (Fernandez-Soto et al., 2016), although it is not yet widely available (La Hoz & Morris, 2019).

Proprietary panels are available for the assessment of gastrointestinal pathogens. BioFire Diagnostics offers an FDA-approved 22-target testing panel for the gastroenteritis, termed the BioFire FilmArray Gastrointestinal Panel. The panel's bacteria targets include *Campylobacter, Clostridium difficile, Plesiomonas shigelloides*, *Salmonella, Yersinia enterocolitica, Vibrio (parahaemolyticus, vulnificus,* and *cholerae),* and *Vibrio cholerae*. The panel's diarrheagenic *E. coli* and *Shigella* targets include Enteroaggregative *E. coli*, Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, Shiga-like toxin-producing *E. coli* stx1/stx2, *E. coli* O157, and *Shigella/*Enteroinvasive *E. coli*. The panel's parasite targets include *Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica,* and *Giardia lamblia*. The panel's virus targets include Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, and Sapovirus (I, II, IV, and V) (BioFire, 2023b). The manufacturer claims a sensitivity of 98.5% and specificity of 99.2% for this test and states that results are available within one hour of testing. However, BioFire notes that the test has not been evaluated for immunocompromised patients (BioFire, 2023b).

The FDA-approved xTAG Gastrointestinal Pathogen Panel, developed by Luminex, can simultaneously identify multiple bacterial, viral and parasitic nucleic acids in both fresh and frozen human stool samples. This test can provide results in as little as five hours and can "detect and identify >90% of the causative bacterial, viral, and parasitic agents of gastroenteritis in the same day (Luminex, 2023b)." The xTAG Gastrointestinal Pathogen Panel is able to identify *Campylobacter*, *Clostridium difficile*, Toxin A/B, *Escherichia coli* O157, *Enterotoxigenic E.coli* (ETEC) LT/ST, Shiga-like Toxin producing *E.coli* (STEC) stx1/stx2, *Salmonella*, *Shigella*, *Vibrio cholerae*, *Yersinia enterocolitica*, Adenovirus 40/41, Norovirus GI/GII, Rotavirus A, *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia* (Luminex, 2023b).

The Biocode Gastrointestinal Pathogen Panel is also an FDA approved test that uses a 96-well microplate to simultaneously detect 17 diarrhea causing pathogens (*Campylobacter*, *Clostridium difficile* toxins A and B, *E. coli* O157, Enterotoxigenic *E. coli* LT/ST (ETEC), Enteroaggregative E. coli (EAEC), Salmonella,

Shiga-like toxin producing E. coli stx1/stx2, Shigella/ Enteroinvasive E. coli, Vibro/ Vibro *parahemolyticus*, *Yersinia enterocolitica*, Adenovirus 40/41, Norovirus GI/GII, Rotavirus A, *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia lamblia*) in stool samples (BioCode, 2023a). This rapid multiplex screening assay is low cost and may be helpful with infection control.

Respiratory Pathogen Panel

Upper respiratory tract infections (involving the nose, sinuses, larynx, pharynx and large airways) can be caused by a variety of viruses and bacteria. These infections may lead to several different patient ailments such as the common cold, acute bronchitis, influenza and respiratory distress syndromes. Regarding the common cold, the most common virus is rhinovirus; the bacteria that most commonly causes a sore throat (pharyngitis) is *Streptococcus pyogenes* (Thomas & Bomar, 2020). Lower respiratory tract infections occur in the lungs and any airways below the larynx. Lower respiratory infections include pneumonia, bronchitis, tuberculosis and bronchiolitis (Hansen, Lykkegaard et al., 2020).

Traditional methods used for the diagnosis of viral respiratory tract infections are direct antigen testing (non-immunofluorescent and immunofluorescent methods) and conventional and rapid cell culture (Ginocchio, 2007). These tests have several limitations including a slow turnaround time, low sensitivity, and labor-intensive processes. Acute respiratory infections may also be diagnosed by a simple respiratory exam, where the physician focuses on the patient's breathing and checks for fluid and inflammation in the lungs. Symptoms of a respiratory tract infection may include a stuffed nose, cough, fever, sore throat, headache, and difficulty breathing. Chest X-rays may be used to check for pneumonia, and blood/mucus samples may be used to confirm the presence of certain bacteria and/or viruses via cell culture. The doctor may also check the ears, nose and throat. Treatment typically incorporates over the counter medications, rest, fluids and antibiotics (if a bacterial infection is identified).

Considerable progress has been made in the development of molecular methods to detect multiple respiratory pathogens simultaneously. Molecular detection, including multiplex PCR assays, is currently the gold standard for viral respiratory diagnosis (Bonnin et al., 2016). Multiplex PCR-based assays are now commercially available to detect several viral pathogens like adenovirus, influenza A and respiratory syncytial virus as well as bacterial pathogens like *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila*. These tests are rapid, sensitive, specific, and the preferred testing method to identify most respiratory pathogens (Caliendo, 2011; Pammi, 2023; Yan et al., 2011). These tests may be a more reliable diagnostic test as they can be performed in just hours, do not require as large a volume of blood, and are not affected by antepartum antibiotics (Pammi, 2023).

BioFire has updated their FDA approved respiratory panel tests, the FilmArray RP and RP2, to become the FilmArray RP2.1 panel test. The new test, RP2.1, has added SARS-CoV-2 as a target compared to the previous versions of the respiratory panels (BioFire, 2023d). The prior FilmArray RP2 was able to detect 17 viral (Adenovirus, Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, Severe Acute Respiratory Syndrome Coronavirus 2, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus) and 4 bacterial (*Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma*

pneumoniae) targets. This FilmArray RP2.1 panel test can now detect 22 targets in 45 minutes with a 97.1% sensitivity and 99.3% specificity (BioFire, 2023d).

GenMark Diagnostics has developed FDA-approved rapid ePlex® Respiratory Pathogen Panel (RP) and Respiratory Pathogen Panel 2 (RP2) tests. They can identify the most common bacterial and viral pathogens causing upper respiratory infections. The RP test can detect pathogens including Adenovirus, Coronavirus (229E, HKU1, NL63, OC43), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A H1, Influenza A H1-2009, Influenza A H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. The RP2 test will detect the same pathogens along with SARS-CoV-2 (GenMark, 2020b). The ePlex® Respiratory Pathogen Panel test was more efficient than a laboratory developed PCR assay resulting "in a significant decrease in time to result, enabling a reduction in isolation days in half of the patients," and increasing the identification of the causative pathogen (van Rijn et al., 2018).

The BioCode Respiratory Pathogen Panel is the FDA approved low-cost test that can simultaneously detect respiratory pathogens in nasopharyngeal swabs. This test is designed in a 96-well microplate format. The following 14 pathogens can be identified with this panel: Adenovirus, Coronavirus (229E, OC43, HKU1, and NL63), Human Metapneumovirus A/B, Influenza A, including subtypes H1, H1 2009 Pandemic, and H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus A/B, Rhinovirus/Enterovirus, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* (BioCode, 2023b).

The NxTAG Respiratory Pathogen Panel, developed by Luminex, is able to simultaneously detect 20 pathogens (Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Rhinovirus/Enterovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Metapneumovirus, Adenovirus, Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, Human Bocavirus, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*) in a single test. The CE Marked panel also detects *Legionella pneumophila* (Luminex, 2023a).

QIAGEN Science has developed the QIAstat-Dx Respiratory SARS-CoV-2 Panel, which is authorized by the FDA under an Emergency Use Authorization (EUA). It can detect the SARS-CoV-2 virus along with 20 other respiratory pathogens, including Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus A+B, Influenza A, Influenza A H1, Influenza A H3, Influenza A H1N1/pdm09, Influenza B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Rhinovirus/Enterovirus, Respiratory Syncytial Virus A+B, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. It is able to provide qualitative results within an hour and is for in vitro diagnostic use (QIAGEN, 2023). When compared with the currently WHO-recommended RT-PCR (WHO-RT-PCR), the QIAstat-Dx Respiratory Panel had a 97% agreement with the WHO-RT-PCR and a sensitivity of 100% and specificity of 93% (Visseaux et al., 2020).

Central Nervous System Panel

The brain is well protected from microbial invasion via the blood-brain barrier (BBB) and blood-

cerebrospinal fluid barrier (BCSFB). Nonetheless, bacteria, fungi, viruses, and amoebae can infect the brain and the consequences are often fatal. Points of entry include the BBB, BCSFB, and the olfactory and trigeminal nerves (Dando et al., 2014). Meningitis, which is when the brain and/or spinal cord become inflamed, is typically caused by viral infections due to enteroviruses; other neurotropic viruses include herpes simplex viruses, human cytomegalovirus, varicella-zoster virus, and rabies virus (Dando et al., 2014). In the United States, bacterial meningitis is most commonly caused by *Streptococcus pneumoniae*, group B *Streptococcus, Neisseria meningitidis, Haemophilus influenzae, Listeria monocytogenes, and Escherichia coli (CDC, 2021a)*. Fungal meningoencephalitis, which is described as inflammation of the brain and surrounding membranes, is often caused by *Cryptococcus, Histoplasma, Blastomyces, Coccidioides, and Candida (CDC, 2021b).* Meningococcal meningitis is typically caused by *Neisseria meningitidis (CDC, 2022b)*. Other types of pathogens may enter the central nervous system. The increasing use of molecular tests for the detection of pathogens in cerebrospinal fluid (CSF) has redefined the diagnosis and management of central nervous system (CNS) infections such as meningitis and encephalitis. However, it is important that test results correlate to the probability of infection. According to Petti and Polage (2019), the number of false-positive test results increase when the multiplex PCR tests are ordered in the absence of an elevated leukocyte count or elevated protein level in the CSF. Hence, the predictive value of the test increases when the tests are ordered only for those patients with a moderate to high pretest probability of having CNS infections based on clinical presentation and CSF findings (Petti & Polage, 2023).

The evaluation of meningitis routinely includes molecular testing, particularly when the patient is suspected to have viral meningitis. Although use of Gram stain and culture is the gold standard for diagnosis of bacterial meningitis, multiplex PCR assays may be useful as an adjunct, especially in patients who have already received antibiotic treatment. Other lab findings (for example, CSF cell count, glucose, and protein analyses) should be used as a screening method prior to the performance of molecular testing. Molecular assays for meningitis caused by fungi, parasites, rickettsia, and spirochetes are in development at this time (Petti & Polage, 2023).

Similarly, molecular testing of CSF is recommended when viral encephalitis, especially encephalitis due to Herpesviridae, is suspected. For other viral encephalitis, the clinical sensitivity and predictive value of multiplex-PCR assays is unknown. Therefore, a negative result does not exclude infection, and a combined diagnostic approach, including other methods like serology, may be necessary to confirm the diagnosis. Multiplex PCR-based assays may be useful in certain cases of bacterial meningitis, especially when a slow-growing or uncultivable bacterium like *Coxiella burnetti* is involved. Molecular assays for encephalitis caused by fungi, parasites, rickettsia, and spirochetes need to be investigated further and are not routinely available at this time (Petti & Polage, 2023).

The FDA approved BioFire FilmArray meningitis/encephalitis panel can provide information on 14 different pathogens in one hour. This test uses 0.2 mL of cerebrospinal fluid, and is able to detect bacteria (*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*), viruses (Cytomegalovirus, Enterovirus, Herpes simplex virus 1, Herpes simplex virus 2, Human herpesvirus 6, Human parechovirus, and Varicella zoster virus) and yeast (*Cryptococcus neoformans/gattii*) (BioFire, 2023c). BioFire states that this panel has an overall sensitivity of 94.2% and a specificity of 99.8% (BioFire, 2023c).

Sepsis Panel

Pathogen Panel Testing, continued

Sepsis, also known as blood poisoning, is the body's systemic immunological response to an infection. Sepsis occurs when an infection (in the lungs, skin, urinary tract or another area of the body) triggers a chain reaction in an individual (CDC, 2021c). Sepsis can lead to end-stage organ failure and death. Septic shock occurs when sepsis results in extremely low blood pressure and abnormalities in cellular metabolism. The annual incidence of severe sepsis and septic shock in the United States is 300 per 100,000 people; sepsis is "the most expensive healthcare problem in the United States" (Gyawali et al., 2019).

Sepsis-related mortality remains high, and inappropriate antimicrobial and anti-fungal treatment is a major factor contributing to increased mortality (Liesenfeld et al., 2014). Blood culture is the standard of care for detecting bloodstream infections, but the method has several limitations (Lamy et al., 2020). Fastidious, slow-growing, and uncultivable organisms are difficult to detect by blood culture, and the test sensitivity decreases greatly when antibiotics have been given prior to culture. Additionally, culture and susceptibility testing may require up to 72 hours to produce results. Multiplex PCR assays of positive blood culture bottles have a more rapid turnaround time and are not affected by the administration of antibiotics. Faster identification and resistance characterization of pathogens may lead to earlier administration of the appropriate antibiotic, resulting in better outcomes, and may lessen the emergence of antibiotic-resistant organisms (Banerjee et al., 2015).

The T2Bacteria Panel is the first "FDA-cleared test to identify sepsis-causing bacteria directly from whole blood without the wait for blood culture (T2Biosystems, 2023)." This panel is able to identify 50% of all bloodstream infections, 90% of all ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*) pathogens, and 70% of all blood culture species identified in the emergency room with a 95% sensitivity and 98% sensitivity (T2Biosystems, 2023).

The Magicplex™ Sepsis Real-time Test by Seegene can identify more than 90 sepsis-causing pathogens with only 1 mL of whole blood. This test identifies both bacteria and fungi, as well as three drug resistance markers in only six hours (Seegene, 2020, 2022).

GenMark has developed three ePlex® Blood Culture Identification (BCID) Panels. These include the ePlex BCID-Gram Positive Panel (identifies 20-gram positive bacteria and four resistance genes), the ePlex BCID-Gran Negative Panel (identifies 21-gram negative bacteria and six resistance genes), and the ePlex BCID-Fungal Panel (identifies 15-fungal organisms) (GenMark, 2022a).

BioFire has developed the FDA-cleared FilmArray Blood Culture Identification Panel (BCID). The original panel could identify 24 targets, but the newly expanded BCID2 panel can identify 43 targets. Targets include gram-positive bacteria (*Enterococcus faecalis, Enterococcus faecium, Listeria monocytogenes, Staphylococcus, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, Streptococcus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes*), gram-negative bacteria (*Acinetobacter calcoaceticus-baumannii complex, Bacteroides fragilis, Enterobacterales, Enterobacter cloacae complex, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae group, Proteus, Salmonella, Serratia marcescens, Haemophilus influenzae, Neisseria meningitidis, Pseudomonas aeruginosa, Stenotrophomonas maltophilia*), yeast (*Candida albicans, Candida auris, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis, Cryptococcus*

neoformans/gattii), and antimicrobial resistance genes (BioFire, 2023a).

Urinary Tract Infection Panel

Urinary tract infections (UTIs) occur in the urinary system and can be either symptomatic or asymptomatic. UTIs can include cystitis, an infection of the bladder or lower urinary tract, pyelonephritis, an infection of the upper urinary tract or kidney, urosepsis, urethritis, and male-specific conditions, such as bacterial prostatitis and epididymitis (Bonkat et al., 2023; Hooton & Gupta, 2023). Typically, in an infected person, bacteriuria and pyuria (the presence of pus in the urine) are present and can be present in both symptomatic and asymptomatic UTIs. A urine culture can be performed to determine the presence of bacteria and to characterize the bacterial infection (Meyrier, 2023).

Panels comprising common UTI pathogens are now commercially available. Firms such as MicroGenDX and NovaDX offer panels consisting of many different pathogens involved in UTIs (MicroGenDX, 2019a; NovaDX, 2023). The NovaDX is a qPCR based test which can detect 17 pathogens including bacteria (*Acinetobacter baumannii, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus saprophyticus*, and *Streptococcus agalactiae*) and yeast (*Candida albicans*) (NovaDX, 2023).

Cardwell et al. (2016) evaluated the microbiology of UTIs in hospitalized adults. Approximately 308 patients were included, with a total of 216 identified pathogens. The authors separated patients into three groups; "community acquired (Group 1); recent healthcare exposure (Group 2); or a history of identification of an extended-spectrum beta lactamase (ESBL)-producing organism (Group 3)." *Escherichia coli* was found to be the most common pathogen, but the frequency differed between groups. Other commonly identified pathogens included *Pseudomonas aeruginosa* (Cardwell et al., 2016).

Medina and Castillo-Pino (2019) estimated the prevalence of certain pathogens in UTI (complicated or uncomplicated). The authors found that up to 75% of uncomplicated UTIs and up to 65% of complicated are caused by uropathogenic *Escherichia coli* (UPEC). Other commonly seen pathogens included *Enterococcus spp*, Group B Streptococcus, *K. pneumonia*, and *S. saprophyticus* (Medina & Castillo-Pino, 2019).

Wound Panel

Wounds (acute or chronic) are almost always colonized by microbes, thereby leading to a significant rate of infection. Panel testing many pathogens have been proposed as a method to quickly identify and therefore treat a wound infection (Armstrong & Meyr, 2021). These panels may be culture-based or nucleic acid-based; nucleic acid panels are typically touted for their speed compared to culture panels.

Firms, such as GenetWorx, Viracor, and MicroGenDX, offer comprehensive panels addressing many different common pathogens, resistance genes, and more. Genera, such as *Streptococcus, Enterococcus*, and *Staphlococcus* are frequent targets of these panels. Different combinations of panels are available (GenetWorx, 2023; MicroGenDX, 2019b; Viracor, 2023).

Pathogen Panel Testing, continued

The Wounds Pathogen Panel by GenetWorx can identify 30 targets including bacteria, fungi, and viruses. Targeted pathogens include *Enterococcus faecalis*, *Enterococcus faecium, Methicillin Resistant Staphylococcus aureus* (MRSA), *Methicillin Sensitive Staphylococcus aureus* (MSSA), *Staphylococcus epidermidis, Streptococcus pyogenes* (Group A Strep), *Streptococcus agalactiae* (Group B Strep), *Streptococcus dysgalactiae* (Group C Strep), *Acinetobacter baumannii, Bacteroides fragilis, Bartonella henselea, Bartonella quintana, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Morganella morganii, Proteus mirabilis, Pseudomonas aeruginosa, Bartonella Quintana, Serratia marcescens, Candida albicans, Candida glabrata, Candida parapsilosis, Candida dubliniensis, Candida tropicalis, Candida krusei, Tricophyton metagrophytes, Trichophyton rubrum, Aspergillus fumigatus, Mycobacterium fortuitum*, Herpes Simplex Virus 1, Herpes Simplex Virus 2, and Herpes Simplex Virus 3 (GenetWorx, 2023).

The Viracor Skin and Soft Tissue Infection Panel can identify 19 bacterial targets using TEM-PCRTM (Target Enriched Multiplex Polymerase Chain Reaction). These bacterial targets include *Acinetobacter baumannii, Bacteroides spp., Citrobacter freundii, Clostridium novyi/septicum, Clostridium perfringens, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Kingella kingae*, Klebsiella spp., Morganella morganii, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, MRSA- Meth. resistant S. aureus, Panton-Valentine leukocidin gene, Staphylococcus lugdunensis, Streptococcus pyogenes (Group A) and Pseudomonas aeruginosa. This test has not been approved by the FDA and has a two to three day turnaround time (Viracor, 2023).

Ray et al. (2013) described the incidence and microbiology of skin and soft tissue infections (SSTIs). The authors focused on members of a Northern California health plan, identifying 376262 patients with 471550 SSTIs. Approximately 23% of these infections were cultured, 54% of these cultures were pathogen-positive, and *Staphylococcus aureus* was found in 81% of these specimens. The researchers calculated the rate of diagnosed SSTIs to be 496 per 10000 person-years (Ray et al., 2013).

A comprehensive list of the main commercial pathogen panel tests mentioned above can also be found in the table on the next page. This table was last updated on 3/27/2023.

Pathogen Panel Testing, continued

Commercial Pathogen Panel Tests		
Type of Panel	**Name**	**Pathogens Identified**
Gastrointestinal	BioFire FilmArray Gastrointestinal Panel	22 targets including bacteria, parasites, and viruses
	Luminex xTAG Gastrointestinal Pathogen Panel	15 targets including bacteria, parasites, and viruses
	Biocode Gastrointestinal Pathogen Panel	17 targets including bacteria, parasites, and viruses
Respiratory	BioFire FilmArray Respiratory 2.1 (RP2.1) Panel	22 targets including viruses and bacteria
	GenMark Diagnostics Rapid ePlex® Respiratory Pathogen Panel	17+ targets including viruses and bacteria
	GenMark Diagnostics Rapid ePlex® Respiratory Pathogen 2 Panel	18 targets including viruses and bacteria
	BioCode Respiratory Pathogen Panel	17 targets including viruses and bacteria
	Luminex NxTAG Respiratory Pathogen Panel	20 targets including viruses and bacteria
	QIAGEN Sciences QIAstat-Dx Respiratory Pathogen Panel	20 targets including viruses and bacteria
Central Nervous System	BioFire FilmArray Meningitis/ Encephalitis Panel	14 targets including bacteria, viruses, and yeast
Sepsis	T2Bacteria Panel	5 ESKAPE pathogens and potentially more targets
	Magicplex™ Sepsis Real-time Test	90+ including bacteria and fungi
	GenMark ePlex® Blood Culture Identification Panel (Gram-positive, Gram-negative and fungal)	56 bacteria and fungi
	BioFire Blood Culture	43 targets including bacteria and yeast
Urinary Tract Infection	NovaDX UTI Test	17 targets including bacteria and yeast
Wound	GENETWORx PCR Wound Testing	30 targets including bacteria, fungi, mycobacteria, and viruses
	Viracor Skin and Soft Tissue Infection Panel	19 bacterial targets

Clinical Utility and Validity

Several studies demonstrated the overall high sensitivity and specificity of the gastroenterology pathogen panels (Buss et al., 2015; Claas et al., 2013; Onori et al., 2014). Several studies have also indicated that gastrointestinal pathogen panels are more sensitive than culture, microscopy, or antigen detection, thus illustrating the potential of panels as a diagnostic tool for gastrointestinal infections (Buss et al., 2015; Couturier et al., 2011; Humphrey et al., 2016; Liu et al., 2014; Operario & Houpt, 2011). Zhang and colleagues concluded that using multiplex PCR assays in the work-up of infectious gastroenteritis has the potential to improve the diagnosis (Zhang et al., 2015).

Numerous studies have examined the clinical utility of the BioFire FilmArray GI Panel. Stockmann et al. (2015) focused on comparing the accuracy in detecting etiologic agents, particularly *Clostridioides difficile*, in stool specimen of pediatric patients with diarrhea between the FilmArray GI Panel with various standard laboratory methods performed at the discretion of the physician. They found that "a potential aetiologic agent was identified in 46% of stool specimens by standard laboratory methods and in 65% of specimens tested using the FilmArray GI Panel (P<0.001)." This FilmArray GI Panel was also able to detect concurrent infections by diarrheal pathogens other than *C. difficile*, including norovirus in 12% of supposed *C. difficile*-only testing cases. The FilmArray GI Panel also detected a pathogen in 63% of cases without additional *C. difficile* testing performed, and even detected *C. difficile* in 8% of those cases. These results proved the FilmArray GI Panel to be critical in detecting other diarrheal pathogens, and co-infections with other infectious diarrheagenic agents (Stockmann et al., 2015).

Similar results for the FilmArray GI Panel were found in another study for acute diarrhea. In conducting a prospective study, Cybulski et al. (2018) found that FilmArray detected pathogens at a higher rate than culture and at a faster time (35.3% in 18 hours versus 6.0% in 47 hours). This rapidity and accuracy also allowed patients to receive targeted therapy and facilitated quicker discontinuation of empirical antimicrobial therapy, demonstrating an improved clinical sensitivity with the FilmArray GI Panel when compared to culture (Cybulski et al., 2018). Beal et al. (2018) investigated the impact of submitting patient stool specimen for testing by the FilmArray GI panel ("cases") and compared overall findings with control patients from the year prior. The researchers concluded that this panel contributed to reducing the number of days on antibiotics (1.73 days among cases versus 2.12 days among controls), reducing "average length of time from stool culture collection to discharge" (3.4 days among cases vs 3.9 days among controls), and reducing overall health care cost by $293.61. They also found results like the previous studies on the FilmArray GI panel, with increased comprehensiveness of detectable pathogens, and eliminating unnecessary testing and antibiotic use (Beal et al., 2018).

Axelrad et al. (2019) performed a retrospective comparative analysis of patients who underwent testing with the FilmArray GI panel from 2015-2017 and those who solely underwent conventional stool testing from 2012-2015. The FilmArray GI panel detected more pathogens (29.2% positive cases vs 4.1%), and reduced the need for additional endoscopic procedures and abdominal radiology imaging within 30 days following stool testing, as well as reduced chances of antibiotic prescription within 14 days following stool testing. The amassed literature communicates the great clinical utility and extended benefits from a multiplex PCR panel like the FilmArray GI Panel.

Zhan et al. (2020) performed a comparison of the BioFire FilmArray gastrointestinal panel and the Luminex xTAG Gastrointestinal Pathogen Panel for detecting diarrheal pathogens in China in a total of 243 diarrhea specimens. These two panels were highly consistent in detecting norovirus, rotavirus, and *Campylobacter*, but had low consistency in detecting *Cryptosporidium*, *Salmonella*, *Shiga-toxin* producing *Escherichia coli* (STEC) and enterotoxigenic *Escherichia coli* (ETEC). The BioFire FilmArray panel was found to be more sensitive, but the Luminex xTAG Gastrointesinal Pathogen Panel was more specific. There appeared to be additional concern for how the Luminex xTAG Gastrointesinal Pathogen Panel yielded more false negatives when detecting ETEC as well (Zhan et al., 2020).

Jo et al. (2021) evaluated the use of the BioFire FilmArray gastrointestinal panel for pediatric patients with diarrhea. The authors compared the FilmArray GI panel results to conventional PCR for *E. Coli* and Allplex GI-Bacteria Assay results. 184 stool samples were tested. "The BioFire GI Panel demonstrated a sensitivity of 100% for 12 targets and a specificity of >95% for 16 targets." The authors conclude that the FilmArray GI panel is useful for rapid identification of enteropathogenesis in pediatric patients (Jo et al., 2021).

Truong et al. (2021) investigated pediatric healthcare management before and after BioFire FilmArray gastrointestinal panel results were received. The study included 172 children, 120 of which had positive results. Based on the FilmArray GI panel results, the healthcare management plan changed for 23% of patients, including changes to antibiotic treatments, hospitalizations, room isolations, prescription changes, and test cancelations. The authors conclude that the FilmArray GI panel results impacted healthcare management, especially related to antibiotic treatment (Truong et al., 2021). Yoo at al. (2021) also studied the healthcare management of children with acute diarrhea using the BioFire FilmArray gastrointestinal panel. 182 patients were included in the study. "A significant reduction in antibiotic use was observed in the prospective cohort compared to historical cohort, 35.3% vs. 71.8%; $p < 0.001$), respectively." The authors conclude that, likely due to the high positive rate and rapid reporting, the FilmArray GI panel was clinically beneficial for children, especially in reducing antibiotic use and enabling early precaution and isolation (I. H. Yoo et al., 2021).

Nijhuis et al. (2017) compared the GenMark Diagnostics ePlex Respiratory Pathogen panel with laboratory-developed real-time PCR assays for detecting respiratory pathogens. The study included 343 clinical specimens. The RP panel found an agreement of 97.4% with the real-time PCR assay regarding 464 pathogens found. The RP panel detected 17 more pathogens than the real-time PCR, showing that this panel could improve the efficiency of diagnostic "sample-to-answer testing" and cost-effectiveness, despite potentially costing more (Nijhuis et al., 2017).

van Asten et al. (2021) evaluated the performance of the GenMark Diagnostics ePlex Respiratory Pathogen panel and the QIAGEN Sciences QIAstat-Dx Respiratory Pathogen panel. The authors specifically studied the detection of three bacterial targets: *Legionella pneumophila, Mycoplasma pneumoniae* and *Bordetella pertussis*. The study included 56 specimens taken form the lower respiratory tract, five of which were negative and the other 51 had previously tested positive on real-time PCR assays for the targets. "The QIAstat-Dx Respiratory Panel V2 (RP) assay detected all the *L. pneumophila* and *B. pertussis* positive samples but only 11/15 (73.3 %) of the *M. pneumoniae* targets. The ePlex Respiratory Pathogen Panel (RPP) assay detected 10/14 (71.4 %) of the L. pneumophila targets, 8/12 (66.7 %) of the B. pertussis positive samples and 13/15 (86.7 %) of the M. pneumoniae targets." The

authors concluded that the clinical performance of both panels depend on the bacterial lode and sample type (van Asten, Boers, de Groot, Schuurman, & Claas, 2021).

Mormeneo Bayo et al. (2022) compared real-time PCR with microscopy in detecting intestinal protozoa in children. The study used the Seegene Allplex Gastrointestinal panel for the real-time PCR. 500 stool samples were analyzed from children, 15 years of age and under, and grouped into two classifications based on if the children had or had not had clinical parasitosis. Based on microscopy, 6.2% of samples were positive. Based on real-time PCR, 51.2% of samples were positive. The authors concluded that "real-time PCR increases the detection of intestinal protozoa, being underdiagnosed by microscopy, especially D. fragilis, in which PCR is considered the most appropriate method for its detection" (Mormeneo Bayo et al., 2022).

Trujillo-Gómez (2022) studied the diagnostic test accuracy of the FilmArray Meningitis/Encephalitis panel. The authors perfmored a systematic review of 19 studies containing a total of 11,251 participants, and performed a random-effects bivariate meta-analysis of diagnostic test accuracy. Using CSF/blood samples, the sensitivity was estimated to be 89.5% and the specificity was estimated to be 97.4%. Using the "final diagnosis adjudication based on clinical/laboratory criteria" the sensitivity was estimated to be 92.1% and the specificity was estimated to be 99.2%. The authors note that the certainty of evidence was low. The authors conclude that the FilmArray Meningitis/Encephalitis panel "may have acceptable-to-high sensitivities and high specificities for identifying bacteria, especially for *S.pneumoniae*, and viruses, especially for HSV-2, and enteroviruses" but suboptimal sensitivities for *L.monocytogenes, H.influenzae, E.coli*, and HSV-1 (Trujillo-Gómez et al., 2022).

Yoo et al. (2019) compared the Seegene Allplex Gastrointestinal, Luminex xTAG Gastrointestinal Pathogen Panel, and BD MAX Enteric Assays in an effort to determine which was the most efficient in detecting gastrointestinal pathogens from clinical stool samples. A total of 858 stool samples were used in this study. "The overall positive percentage agreements of Seegene, Luminex, and BD MAX were 94% (258 of 275), 92% (254 of 275), and 78% (46 of 59), respectfully. For Salmonella, Luminex showed low negative percentage agreement because of frequent false positives (n = 31) showing low median fluorescent intensity. For viruses, positive/negative percentage agreements of Seegene and Luminex were 99%/96% and 93%/99%, respectively (Yoo et al., 2019)." Overall, the authors conclude by suggest that these assays are promising in the detection of gastrointestinal pathogens simultaneously.

Mahony et al. concluded that multiplex PCR-based testing was the most cost-effective strategy for the diagnosis of respiratory virus infections in children and resulted in better patient outcomes (shorter hospital stays) at lower costs (Mahony et al., 2009). Ginocchio et al. (2009) compared the sensitivities, specificities, positive predictive values, and negative predictive values of four different Influenza A diagnostic tests, including rapid antigen, direct immunofluorescence, viral culture, and PCR panel. The authors inferred that the PCR panel test provided the best diagnostic option with the highest sensitivity for the detection of all influenza strains and identified a significant number of additional respiratory pathogens (Ginocchio et al., 2009). Subramony et al. (2016) reported the use of multiplex PCR-based assays for respiratory viruses in hospitalized patients resulted in decreased healthcare resource utilization, including decreased use of antibiotics and chest radiographs (Subramony et al., 2016). Babady et al. (2018) evaluated a new panel of 19 viruses and two bacteria (ePlex Respiratory Panel) with 2908 samples by comparing it to BioFire FilmArray. Overall agreement was >95% for all targets, and

positive agreement ranged from 85.1% to 95.1%. Negative agreement ranged from 99.5% to 99.8% (Babady et al., 2018).

The Infectious Diseases Society of America (IDSA) stated that CSF RT-PCR can be one of the methods used for the diagnosis of rabies virus and enteroviral encephalitis (Tunkel et al., 2008). Several studies have evaluated the clinical impact of RT-PCR for the detection of enterovirus in the CSF of patients with aseptic meningitis (Ramers et al., 2000; Robinson et al., 2002; Stellrecht et al., 2002). These studies showed a reduction in unnecessary diagnostic and therapeutic intervention (for example, antibiotic use, ancillary tests, etc.), length of hospital stay, and hospital costs. Tzanakaki et al. (2005) evaluated a multiplex PCR assay for detection of *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b, and concluded that the test had high sensitivity (between 88% and 93.9%), an overall specificity and positive predictive value of 100%, and a negative predictive value >99% (Tzanakaki et al., 2005). Leber et al. (2016) evaluated the performance of a commercially available multiplex PCR-based panel for meningitis and encephalitis and concluded that the test is a sensitive and specific aid in diagnosis of CNS infections and leads to improved patient outcomes (Leber et al., 2016). Another study compared the FilmArray meningitis/encephalitis (ME) panel by BioFire Diagnostics, which uses 0.2 mL of CSF to test for 14 pathogens in one hour (BioFire, 2023c), to traditional culture and PCR assay methods. The FilmArray ME panel "demonstrated an overall percent positive agreement (PPA) of 97.5% (78/80) for bacterial pathogens, 90.1% (145/161) for viruses, and 52% (26/50) for *Cryptococcusneoformans/C. gattii*. Despite the low overall agreement (52%) between the ME panel and antigen testing for detection of *C. neoformans/C. gattii*, the percent positive agreement of the FilmArray assay for *C. neoformans/C. gattii* was 92.3%" (Liesenfeld et al., 2014; Liesman et al., 2018). The ME panel has also been proven to aid in "decreas[ing] the utilization of antibiotic therapy among pediatric patients admitted for concerns related to meningitis or encephalitis" (McDonald et al., 2020). Their research demonstrated that introducing the ME panel helped to reduce the days of therapy (DoT) from 5 days to 3 days and the number of inpatient days. Using the ME panel also decreased the empiric use of intravenous third generation cephalosporins and ampicillin for treatment independent of a respiratory viral pathogen diagnosis. Identifying the specific etiology guided more appropriate antibiotic therapy (McDonald et al., 2020).

The use of multiplex PCR assays to identify pathogens following positive blood culture can be faster than standard techniques involving phenotypic identification and antimicrobial susceptibility testing that is required up to 72 hours after the blood culture became positive (Liesenfeld et al., 2014). A prospective randomized controlled trial evaluating outcomes associated with multiplex PCR detection of bacteria, fungi, and resistance genes directly from positive blood culture bottles concluded that the testing led to more judicious antibiotic use (Banerjee et al., 2015). A study by Ward and colleagues compared the accuracy and speed of organism and resistance gene identification of two commercially available multiplex-PCR sepsis panels to conventional culture-based methods for 173 positive blood cultures. The researchers discovered that both the assays accurately identified organisms and significantly reduced the time to definitive results (on average, between 27.95 and 29.17 hours earlier than conventional method) (Ward et al., 2015). Another study assessed the diagnostic accuracy of a commercially available multiplex PCR-based assay for detecting infections among patients suspected of sepsis. They concluded that the test had high specificity with a modest sensitivity and had higher rule-in value than the rule-out

value. If the patient had a positive result, a clinician could confidently diagnose sepsis and begin appropriate antimicrobial therapy while avoiding unwanted additional testing (Chang et al., 2013).

There are a few limitations with this type of testing. First, the level, detection or non-detection, of a microorganism does not necessarily imply a diagnosis. The tests can only describe the levels of microorganisms found in the environment, but additional information is required to make a diagnosis. Second, the scope of the 16S rRNA sequencing used in testing may be limited. Differences in regions more specific than rRNA (such as surface antigens or individual toxin genes) cannot be resolved with this test. For example, the test cannot distinguish between a pathogenic *C. difficile* strain and a nonpathogenic one. Moreover, the tests report some of their targets at a genus level only, which means that these targets cannot be differentiated at the species level (Almonacid et al., 2017; Watts et al., 2017). Finally, the PCR technique can introduce errors during the amplification leading to incorrect detection. PCR enzymes may accidentally create "artefacts" or otherwise incorrect sequences causing the detection or measurement of the microorganisms to be inaccurate (V. Wintzingerode et al., 1997).

UroSwab is a urine-based proprietary test from Medical Diagnostics LLC. UroSwab is a real-time PCR test intended to detect numerous pathogens potentially involved in sexually transmitted and urological infections. This test uses a patient's urine, and the turnaround time is estimated at 24-72 hours. The results include whether a pathogen's presence was normal or abnormal and includes comments on what the pathogen's presence means (Diagnostics, 2015a, 2015b).

McCarty et al. (2023) tested the performance and clinical utility of the GenMark ePlex Blood Culture Identification Gram-Negative Panel. The authors used "matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry on bacterial isolates" as a reference to compare results. In total, 98.1% (106/108) of the bacteria identified by MALDI were on the GenMark panel, and "valid tests (107/108, 99.1%) yielded results on average 26.7 h earlier" (McCarty et al., 2023).

IV. Guidelines and Recommendations

American College of Gastroenterology (ACG)

American College of Gastroenterology (ACG) stated that "diarrheal disease by definition has a broad range of potential pathogens particularly well suited for multiplex molecular testing. Several well-designed studies show that molecular testing now surpasses all other approaches for the routine diagnosis of diarrhea. Molecular diagnostic tests can provide a more comprehensive assessment of disease etiology by increasing the diagnostic yield compared with conventional diagnostic tests (Riddle et al., 2016)." Furthermore, the ACG recommended that "traditional methods of diagnosis (bacterial culture, microscopy with and without special stains and immunofluorescence, and antigen testing) fail to reveal the etiology of the majority of cases of acute diarrheal infection. If available, the use of Food and Drug Administration-approved culture independent methods of diagnosis can be recommended at least as an adjunct to traditional methods. (Strong recommendation, low level of evidence)" (Riddle et al., 2016)

The ACG also notes:

- "Diagnostic evaluation using stool culture and culture-independent methods if available should be used in situations where the individual patient is at high risk of spreading disease to others, and during known or suspected outbreaks."
- "Stool diagnostic studies may be used if available in cases of dysentery, moderate–severe disease, and symptoms lasting >7 days to clarify the etiology of the patient's illness and enable specific directed therapy" (Riddle et al., 2016).

In 2013, the ACG made the following recommendations on diagnostic tests used for Clostridium difficile infections (Surawicz et al., 2013):

- "Only stools from patients with diarrhea should be tested for Clostridium difficile. (Strong recommendation, high-quality evidence)"
- "Nucleic acid amplification tests (NAAT) for C. difficile toxin genes such as PCR are superior to toxins A + B EIA testing as a standard diagnostic test for CDI. (Strong recommendation, moderate-quality evidence)"
- "Glutamate dehydrogenase (GDH) screening tests for C difficile can be used in two- or three-step screening algorithms with subsequent toxin A and B EIA testing, but the sensitivity of such strategies is lower than NAATs. (Strong recommendation, moderate-quality evidence)"
- "Repeat testing should be discouraged. (Strong recommendation, moderate-quality evidence)"
- "Testing for cure should not be done. (Strong recommendation, moderate-quality evidence)"

Infectious Diseases Society of America (IDSA)

In 2013, the IDSA stated that "molecular diagnostics that detect microbial DNA directly in blood have achieved a modest level of success, but several limitations still exist. Based on available data, well-designed multiplex PCRs appear to have value as sepsis diagnostics when used in conjunction with conventional culture and routine antibiotic susceptibility testing" (Caliendo et al., 2013).

The IDSA published guidelines for the diagnosis and management of infectious diarrhea which state:

Stool testing should be performed for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *C. difficile*, and STEC in people with diarrhea accompanied by fever, bloody or mucoid stools, severe abdominal cramping or tenderness, or signs of sepsis. However, other bacterial, viral, and parasitic agents should be considered regardless of symptoms. Any specimen testing positive for bacterial pathogens by culture independent diagnostics (such as an antigen based molecular assay) should be cultured in a clinical or public health laboratory if isolation was requested or required. Finally, clinical consideration should occur with interpretation of results of multi-pathogen NAATs as these tests only detect DNA and not necessarily pathogens (Shane et al., 2017).

The IDSA acknowledges the availability of an FDA-approved multiplex PCR targeting 14 organisms for diagnosing encephalitis and meningitis, but the society states it "should not be considered a replacement for culture." The IDSA also notes that for gram-negative or gram-positive bacteria, bacterial culture is noted as the main diagnostic procedure (albeit at low sensitivity and optional). Regarding UTI, the IDSA only recommends nucleic acid testing for adenovirus and BK polyoma virus (Miller et al., 2018).

Regarding "wounds" (termed skin and soft tissue infections in the IDSA guideline), the IDSA typically recommends culture for most pathogens. Only a few strains of bacteria and viruses (such as *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus spp,* MRSA, and streptococci) were recommended for nucleic acid testing with the majority of bacterial and fungal pathogens recommended for culture instead (Miller et al., 2018).

The IDSA recommends RT-PCR or other molecular tests over other influenza tests in hospitalized patients. RT-PCR tests targeting a panel of respiratory pathogens are recommended in hospitalized, immunocompromised patients (Uyeki et al., 2018).

Global Wound Biofilm Expert Panel Consensus Guidelines

A Global Wound Biofilm Expert Panel have strongly agreed that "there are currently no routine diagnostic tests available to confirm biofilm presence" and that "the most important measure for future diagnostic tests to consider is indication of where the biofilm is located within the wound (Schultz et al., 2017)."

Society of Critical Care Medicine and the European Society of Intensive Care Medicine (SCCM)

A joint collaboration of the Society of Critical Care Medicine and the European Society of Intensive Care Medicine issued international guidelines for management of sepsis and septic shock. It states "in the near future, molecular diagnostic methods may offer the potential to diagnose infections more quickly and more accurately than current techniques. However, varying technologies have been described, clinical experience remains limited, and additional validation is needed before recommending these methods as an adjunct to or replacement for standard blood culture techniques (Rhodes et al., 2017)."

A 2020 update regarding "Management of Septic Shock and Sepsis-Associated Organ Dysfunction in Children" was published by the Society of Critical Care Medicine (SCCM), European Society of Intensive Care Medicine (ESICM), and the International Sepsis Forum. In it, they acknowledge the presence of new molecular technologies, but remark that they are "currently relatively expensive, are not sufficient for all pathogens and antibiotic sensitivities, and are not universally available" (Weiss et al., 2020).

National Institute for Health and Care Excellence (NICE)

NICE states there is "insufficient evidence to recommend the routine adoption in the NHS of the integrated multiplex polymerase chain reaction tests, xTAG Gastrointestinal Pathogen Panel, FilmArray GI Panel and Faecal Pathogens B assay, for identifying gastrointestinal pathogens in people with suspected gastroenteritis." NICE acknowledges that the tests show promise but need further data on their clinical utility (NICE, 2017).

American Society for Microbiology/Association for Molecular Pathology/Association of Public Health Laboratories/College of American Pathologists/Infectious Diseases Society of America/Pan American Society for Clinical Virology

These societies made a joint statement regarding respiratory viral panels and noted three populations in which multiplex panels would be beneficial. Those populations were "immunocompromised hosts, adult patients appearing acutely ill who are potential hospital admissions, and critically-ill adult patients, particularly ICU patients" (American Society for Microbiology, 2017).

American College of Chest Physicians (CHEST)

The CHEST has recommended that outpatient adults with an acute cough and suspected pneumonia should not undergo routine microbiological testing because there is no need for such testing. However, testing may be considered if the results would change the therapeutic approach. Microbiological tests may include culture, serologic, and PCR testing (Hill et al., 2019).

Centers for Disease Control and Prevention (CDC)

Regarding molecular tests that are commonly used for a *C. difficile* diagnosis, the CDC states that "FDA-approved PCR assays, which test for the gene encoding toxin B, are same-day tests that are highly sensitive and specific for the presence of a toxin-producing *C. diff* organism. Molecular assays can be positive for *C. diff* in individuals who are asymptomatic. When using multi-pathogen (multiplex) molecular methods, the results should be read with caution. In addition, patients with other causes of diarrhea might be positive, which could lead to over-diagnosis and treatment." (CDC, 2020)

Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA)

The IDSA and SHEA have stated that the best-performing method for detecting patients with a greater risk of a *C. difficile* infection from a stool sample is to "Use a stool toxin test as part of a multistep algorithm (ie, glutamate dehydrogenase [GDH] plus toxin; GDH plus toxin, arbitrated by nucleic acid amplification test [NAAT]; or NAAT plus toxin) rather than a NAAT alone for all specimens received in the clinical laboratory when there are no preagreed institutional criteria for patient stool submission (Figure 2) (weak recommendation, low quality of evidence) (L. C. McDonald et al., 2018)." These guidelines also state that repeat testing (within 7 days) should not be performed. Panel testing is not specifically mentioned in these guidelines.

The European Association of Urology (EAU)

The EAU published urological infections guidelines. For uncomplicated UTIs (recurrent UTIs, cystitis, pyelonephritis), the EAU does not mention molecular testing at any point of the treatment algorithm; instead, they recommend bacterial culture or dipstick testing for diagnosis and recommending against extensive workup. The EAU notes that antimicrobial susceptibility testing should be performed in all cases of pyelonephritis, but their guidelines do not suggest any methods over another. In complicated UTIs, the EAU recommends urine culture to identify cases of clinically significant bacteriuria (Bonkat et al., 2023).

American Society of Transplantation Infectious Diseases Community of Practice

These guidelines focus on identifying infections in transplant patients. Their recommendations are as follows:

"For the diagnosis of SOT [solid organ transplant] recipients with suspected gastrointestinal infections", gastrointestinal multiplex molecular assays are recommended to identify *Cryptosporidium, Cyclospora,* and *Giardia* (La Hoz & Morris, 2019).

American Society for Clinical Pathology (ASCP, through ChoosingWisely)

The ASCP states "Do not routinely order broad respiratory pathogen panels unless the result will affect patient management." They further state that patient management may include "provid [ing] immediate diagnosis and potentially expedite management decisions" and list "rapid molecular or point of care tests for RSV, Influenza A/B, or Group A pharyngitis" as examples (ASCP, 2019a).

The ASCP recommends against testing "for community gastrointestinal stool pathogens in hospitalized patients who develop diarrhea after day 3 of hospitalization" and instead recommend considering testing for *C. difficile* (ASCP, 2019b).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.
All the below descriptions are taken from the FDA website.

Respiratory Pathogen Panels

On January 10, 2011, the FDA approved the Verigene® Respiratory Virus Plus Nucleic Acid Test (RV+) on the Verigene® System as a qualitative nucleic acid multiplex test intended to simultaneously detect and identify multiple respiratory virus nucleic acids in nasopharyngeal (NP) swab specimens from individuals with signs and symptoms of respiratory tract infection.

On February 17, 2012, the FDA approved the xTAG® Respiratory Viral Panel (RVP) as a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasopharyngeal swabs from individuals suspected of respiratory tract infections.

On September 10, 2012, the eSensor Respiratory Viral Panel (RVP) was approved as a qualitative nucleic acid multiplex in vitro diagnostic test intended for use on the eSensor XT-8 system for the simultaneous detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory infection.

On December 17, 2015, the FDA approved NxTAG® Respiratory Pathogen Panel as a qualitative test intended for use on the Luminex® MAGPIX® Instrument for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria extracted from nasopharyngeal swabs collected from individuals with clinical signs and symptoms of a respiratory tract infection.

On May 30, 2017, the FDA approved the FilmArray® Respiratory Panel 2 (RP2), a multiplexed nucleic acid test intended for use with FilmArray® 2.0 or FilmArray® Torch systems for the simultaneous qualitative

detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections.

On June 9, 2017, the FDA approved the EPlex Respiratory Pathogen Panel as a multiplexed nucleic acid in vitro diagnostic test intended for use on the ePlex® Instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory tract infection.

On August 30, 2017, the FDA approved the Idylla Respiratory (IFV-RSV) Panel, which is an in vitro assay intended for the qualitative detection of nucleic acids for Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1, H275Y mutation of Influenza A subtype 2009 H1, Influenza B and Respiratory Syncytial Virus (A and B) from nasopharyngeal swabs in viral transport media of adult and pediatric patients. The test uses the Idylla system to aid in the diagnosis of respiratory viral infection when used in conjunction with other clinical and laboratory findings.

On March 30, 2020, under emergency use authorization, the FDA approved the QIAstat-Dx Respiratory SARS-CoV-2 Panel as a multiplexed nucleic acid real-time PCR test intended for the qualitative detection and differentiation of nucleic acid from multiple respiratory viral and bacterial organisms, including the SARS-CoV-2 virus, in nasopharyngeal swabs (NPS) eluted in universal transport media collected from patients suspected of COVID-19 by their healthcare provider.

On October 8, 2020, under emergency use authorization, the FDA approved the EPlex Respiratory Pathogen Panel 2 as a multiplexed nucleic acid in vitro diagnostic test t intended for use on the ePlex Instrument for the simultaneous qualitative detection and differentiation of nucleic acids from multiple respiratory viral and bacterial organisms, including nucleic acid from Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), in nasopharyngeal swabs (NPS) eluted in viral transport media obtained from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider.

On March 17, 2021, under emergency use authorization, approved the FilmArray® Respiratory Panel 2.1 (RP2.1), which is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and differentiation of nucleic acids from multiple viral and bacterial respiratory organisms, including nucleic acid from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), in nasopharyngeal swabs (NPS) obtained from individuals suspected of COVID-19 by their healthcare provider.

Blood Culture Pathogen Panels

On January 30, 2015, the FDA approved FilmArray Blood Culture Identification (BCID) Panel for use with the FilmArray 2.0.

On March 25, 2016, the FDA approved the Great Basin Staph ID/R Blood Culture Panel is a qualitative, multiplex, nucleic acid-based in vitro diagnostic assay intended for the simultaneous identification of nucleic acid from Staphylococcus aureus, Staphylococcus lugdunensis and various Staphylococcus species to the genus level and the detection of the mecA gene for methicillin resistance directly from patient positive blood culture specimens.

On June 22, 2017, the FDA approved FilmArray NGDS Warrior Panel.

Meningitis Pathogen Panels

On October 8, 2015, the FDA approved the FilmArray Meningitis/Encephalitis (ME) Panel as a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with FilmArray and FilmArray 2.0 systems. The FilmArray ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis.

Gastrointestinal Pathogen Panels

On January 16, 2013, the FDA approved the Prodesse® ProGastro SSCS Assay as a multiplex real time PCR in vitro diagnostic test for the qualitative detection and differentiation of *Salmonella*, *Shigella*, and Campylobacter (*C. jejuni* and *C. coli* only, undifferentiated) nucleic acids and Shiga Toxin 1 (stx1) and Shiga Toxin 2 (stx2) genes. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2. Nucleic acids are isolated and purified from preserved stool specimens obtained from symptomatic patients exhibiting signs and symptoms of gastroenteritis.

On March 21, 2013, the FDA approved the xTAG® Gastrointestinal Pathogen Panel (GPP) as a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis.

On May 2, 2014, the FDA approved the FilmArray Gastrointestinal (GI) Panel as a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with the FilmArray Instrument. The FilmArray GI Panel is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection.

On June 20, 2014, the FDA approved the Verigene Enteric Pathogens Nucleic Acid Test (EP) as a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria and genetic virulence markers from liquid or soft stool preserved in Cary-Blair media, collected from individuals with signs and symptoms of gastrointestinal infection.

On September 16, 2014, the FDA approved the e xTAG® Gastrointestinal Pathogen Panel (GPP) as a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, bacterial and parasitic nucleic acids in human stool specimens or human stool in Cary Blair media from individuals with signs and symptoms of infectious colitis or gastroenteritis.

On May 2, 2017, the FDA approved the BD MAX Extended Enteric Bacterial Panel performed on the BD MAX System, as an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens.

On July 12, 2017, the FDA approved the Great Basin Stool Bacterial Pathogens Panel is a multiplexed, qualitative test for the detection and identification of DNA targets of enteric bacterial pathogens. The Stool Bacterial Pathogens Panel is performed directly from Cary Blair or C&S Medium preserved stool

specimens from symptomatic patients with suspected acute gastroenteritis, enteritis, or colitis and is performed on the Portrait™ Analyzer.

On November 29, 2018, the FDA approved the BD Max Enteric Viral Panel for use as an in vitro diagnostic test to detect and differentiate enteric viral pathogens, including Norovirus, Rotavirus, Adenovirus, Sapovirus, and human Astrovirus.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
64530	Scraping of cornea, diagnostic, for smear and/or culture
87252	Virus isolation; tissue culture inoculation, observation, and presumptive identification by cytopathic effect
87483	Infectious agent detection by nucleic acid (DNA or RNA); central nervous system pathogen (e.g., Neisseria meningitidis, Streptococcus pneumoniae, Listeria, Haemophilus influenzae, E. coli, Streptococcus agalactiae, enterovirus, human parechovirus, herpes simplex virus type 1 and 2, human herpesvirus 6, cytomegalovirus, varicella zoster virus, Cryptococcus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
87505	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (e.g., Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87506	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (e.g., Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets
87507	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (e.g., Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
87631	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (e.g., adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87632	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (e.g., adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets
87633	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (e.g., adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription,

	when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
87636	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) and influenza virus types A and B, multiplex amplified probe technique
87637	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) and influenza virus types A and B, and respiratory syncytial virus, multiplex amplified probe technique
0068U	Candida species panel (C. albicans, C. glabrata, C. parapsilosis, C. kruseii, C tropicalis, and C. auris), amplified probe technique with qualitative report of the presence or absence of each species Proprietary test: MycoDART-PCR™ dual amplification real time PCR panel for 6 Candida species Lab/Manufacturer: RealTime Laboratories, Inc/MycoDART, Inc
0086U	Infectious disease (bacterial and fungal), organism identification, blood culture, using rRNA FISH, 6 or more organism targets, reported as positive or negative with phenotypic minimum inhibitory concentration (MIC)-based antimicrobial susceptibilityProprietary test: Accelerate PhenoTest™ BC kitLab/Manufacturer: Accelerate Diagnostics, Inc.
0109U	Infectious disease (Aspergillus species), real-time PCR for detection of DNA from 4 species (A. fumigatus, A. terreus, A. niger, and A. flavus), blood, lavage fluid, or tissue, qualitative reporting of presence or absence of each species Proprietary test: MYCODART Dual Amplification Real Time PCR Panel for 4 Aspergillus species Lab/Manufacturer: RealTime Laboratories/MycoDART, Inc
0112U	Infectious agent detection and identification, targeted sequence analysis (16S and 18S rRNA genes) with drug-resistance gene Proprietary test: MicroGenDX qPCR & NGS For Infection Lab/Manufacturer: MicroGenDX
0115U	Respiratory infectious agent detection by nucleic acid (DNA and RNA), 18 viral types and subtypes and 2 bacterial targets, amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected Proprietary test: ePlex Respiratory Pathogen (RP) Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0140U	Infectious disease (fungi), fungal pathogen identification, DNA (15 fungal targets), blood culture, amplified probe technique, each target reported as detected or not detectedProprietary test: ePlex® BCID Fungal Pathogens PanelLab/Manufacturer: GenMark Diagnostics, Inc
0141U	Infectious disease (bacteria and fungi), gram-positive organism identification and drug resistance element detection, DNA (20 gram-positive bacterial targets, 4 resistance genes, 1 pan gram-negative bacterial target, 1 pan Candida target), blood culture, amplified probe technique, each target reported as detected or not detected

	Proprietary test: ePlex® BCID Gram-Positive Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0142U	Infectious disease (bacteria and fungi), gram-negative bacterial identification and drug resistance element detection, DNA (21 gram-negative bacterial targets, 6 resistance genes, 1 pan gram-positive bacterial target, 1 pan Candida target), amplified probe technique, each target reported as detected or not detected Proprietary test: ePlex® BCID Gram-Negative Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0152U	Infectious disease (bacteria, fungi, parasites, and DNA viruses), DNA, PCR and next-generation sequencing, plasma, detection of >1,000 potential microbial organisms for significant positive pathogens Proprietary test: Karius® Test Lab/Manufacturer: Karius Inc
0240U	Infectious disease (viral respiratory tract infection), pathogen-specific RNA, 3 targets (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], influenza A, influenza B), upper respiratory specimen, each pathogen reported as detected or not detected Proprietary test: Xpert® Xpress SARSCoV-2/Flu/RSV (SARS-CoV-2 & Flu targets only) Lab/Manufacturer: Cepheid
0241U	Infectious disease (viral respiratory tract infection), pathogen-specific RNA, 4 targets (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], influenza A, influenza B, respiratory syncytial virus [RSV]), upper respiratory specimen, each pathogen reported as detected or not detected Proprietary test: Xpert® Xpress SARSCoV-2/Flu/RSV (all targets) Lab/Manufacturer: Cepheid
0321U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 20 bacterial and fungal organisms and identification of 16 associated antibiotic-resistance genes, multiplex amplified probe technique Proprietary test: Bridge Urinary Tract Infection Detection and Resistance Test Lab/Manufacturer: Bridge Diagnostics
0323U	Infectious agent detection by nucleic acid (DNA and RNA), central nervous system pathogen, metagenomic next-generation sequencing, cerebrospinal fluid (CSF), identification of pathogenic bacteria, viruses, parasites, or fungi Proprietary test: Johns Hopkins Metagenomic Next-Generation Sequencing Assay for Infectious Disease Diagnostics Lab/Manufacturer: Johns Hopkins Medical Microbiology Laboratory
0369U	Infectious agent detection by nucleic acid (DNA and RNA), gastrointestinal pathogens, 31 bacterial, viral, and parasitic organisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique
0370U	Infectious agent detection by nucleic acid (DNA and RNA), surgical wound pathogens, 34 microorganisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique, wound swab
0371U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogen, semiquantitative identification, DNA from 16 bacterial organisms and 1 fungal

	organism, multiplex amplified probe technique via quantitative polymerase chain reaction (qPCR), urine
0373U	Infectious agent detection by nucleic acid (DNA and RNA), respiratory tract infection, 17 bacteria, 8 fungus, 13 virus, and 16 antibiotic-resistance genes, multiplex amplified probe technique, upper or lower respiratory specimen
0374U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 21 bacterial and fungal organisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique, urine

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes
4/27/22	Added CPT code 0321U (Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 20 bacterial and fungal organisms and identification of 16 associated antibiotic-resistance genes, multiplex amplified probe technique Proprietary test: Bridge Urinary Tract Infection Detection and Resistance Test Lab/Manufacturer: Bridge Diagnostics), which is a not-covered code with Select Health.
8/22/22	Modified wording in overall criteria to ensure clarity. Also, added CPT codes 0109U, 0323U, and 0330U.
3/20/23	Added coverage criteria #8 (In the outpatient setting, molecular detection-based panel testing of pathogens for urinary tract infection, sinusitis, and prostatitis, or other conditions not listed above are not medically necessary, as they are not superior to standard of care testing and **DO NOT MEET COVERAGE CRITERIA.**)

G2149 Pathogen Panel Testing

Pediatric Preventive Screening

Policy #: AHS – G2042 – Pediatric Preventive Screening	**Prior Policy Name & Number (as applicable):** G2042 – Preventive Screening in Children and Adolescents
Implementation Date: 9/15/21	**Date of Last Revision:** 4/29/22, 5/18/23, 10/18/23 (see Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Preventive screening is a healthcare service with the goal of illness prevention and health management. According to the American College of Preventive Medicine (ACPM, 2019) "preventive medicine focuses on the health of individuals, communities, and defined populations. Its goal is to protect, promote, and maintain health and well-being and to prevent disease, disability, and death."

Pediatric preventive screening guidelines provide evidence-driven guidance for preventive care screenings and well-child visits. Bright Futures is a "national health promotion and prevention initiative, led by the American Academy of Pediatrics and supported by the Maternal and Child Health Bureau, Health Resources and Services Administration (AAP, 2021a).

This policy refers to laboratory-based preventive screening tests performed on individuals newborn through age 18 years, except for newborn screening for genetic disorders. The World Health Organization (WHO) defines an adolescent as any person between the age of 10 and 19 (WHO, 2021).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request

1. When it follows all applicable federal and state law recommendations, a newborn screening panel **MEETS COVERAGE CRITERIA**.
2. For all newborns, screening for hyperbilirubinemia **MEETS COVERAGE CRITERIA**.
3. For all newborns, screening for congenital hypothyroidism utilizing serum thyroxine (T4) and/or thyroid-stimulating hormone (TSH) **MEETS COVERAGE CRITERIA**.
4. For all newborns, screening for sickle cell disease **MEETS COVERAGE CRITERIA**.
5. Blood lead screening **MEETS COVERAGE CRITERIA** for any of the following situations:
   a. For individuals ages 12 months to 2 years.
   b. For individuals ages 6 months to 6 years who have an increased risk for lead exposure (see Note 1).
6. Screening for anemia with hemoglobin or hematocrit determination **MEETS COVERAGE CRITERIA** for any of the following situations:
   a. For all individuals who are 12 months of age.
   b. For individuals 4 months and older who are at risk for iron deficiency (see Note 2).
7. For individuals 1 month of age or older who are at increased risk of contracting tuberculosis (see Note 3), tuberculosis screening **MEETS COVERAGE CRITERIA.**
8. Screening for dyslipidemia using a fasting lipid profile or a non-fasting non-HDL-C **MEETS COVERAGE CRITERIA** in any of the following situations:
   a) Annually for children and adolescents who are at increased risk due to personal history or family history (see Note 4).

Notes:

Note 1: Lead exposure risk factors for children as defined by the CDC: living or spending time in a house or building built before 1978; growing up in a low-income household; being a recent immigrant, refugee or recently adopted from less developed countries; living or spending time with a person who works with lead or has hobbies that expose them to lead (CDC, 2022).

Note 2: Iron deficiency risk factors for children as defined by the AAP: history of prematurity or low birth weight; exposure to lead; exclusive breastfeeding beyond 4 months of age without supplemental iron; weaning to whole milk or complementary foods that do not include iron-fortified cereals or foods naturally rich in iron, feeding problems, poor growth, and inadequate nutrition (Baker et al., 2010).

Note 3: TB risk factors for children as defined by the AAP: close contact with a person with or suspected to have infectious tuberculosis; radiographic or clinical findings suggestive of TB; HIV infection or considered at risk for HIV infection; being of foreign birth (especially if born in Asia, Africa or Latin America, countries of the former Soviet Union) or is a refugee, or immigrant; contact with HIV infected, homeless, nursing home residents, institutionalized or incarcerated individuals, illicit drug users or migrant farm

workers; having a depressed immune system; living or has lived in a "high risk for tuberculosis" area; participating in significant travel to countries with endemic infections (AAP, 2022; Nolt et al., 2021).

Note 4: Dyslipidemia risk factors for children as defined by the AAP: pediatric patient family history includes family members with CVD or dyslipidemia that are ≤55 years of age for men and ≤65 years of age for women; pediatric patients who have an unknown family history or other CVD risk factors such as being overweight (BMI ≥ 85th percentile, <95th percentile), obesity (BMI ≥ 95th percentile), hypertension (blood pressure ≥ 95th percentile), cigarette smoking, or diabetes mellitus (Daniels et al., 2008).

III. Scientific Background

The annual "wellness visit" or checkup visit to a primary care provider has been a common component of routine healthcare for several decades. Providers typically review an individual's personal history and family history, perform a physical examination, and run a battery of tests during the annual checkup. The types and number of tests performed can vary widely among providers.

Screening (checking for disease when there are no symptoms) can improve the likelihood of early detection, and therefore, also prognosis (NCI, 2022). The characteristics of a disease or condition, such as significant effects of an untreated disease, high prevalence in healthy populations, and utility of preclinical detection, make a condition a good candidate for screening. Newborns and adolescents are more susceptible to certain conditions than adults, and consequently, are recommended for different screenings. For example, infants are typically screened for hyperbilirubinemia, although this condition is not seen as frequently in older children or adults. Schools will often be responsible for the screening of certain conditions, including scoliosis (Kelly, 2020).

Newborn screening is provided to healthy populations to identify newborns that require further testing. Each state handles newborn screening according to predetermined mandates. The United States Secretary of Health and Human Services has established the Recommended Uniform Screening Panel (RUSP) which provides a list of conditions that should be screened, including cystic fibrosis and phenylketonuria. A blood sample is typically taken from the heel of the newborn around the time of hospital discharge (Kemper, 2020). Most of these conditions are identified with tandem mass spectrometry or high pressure liquid chromatography, which are both well-validated HRSA, 2018).

Screening in children and adolescents is also critical. Some of these screenings may not have apparent benefits for many years or even until adulthood, and the American Academy of Pediatrics (AAP) emphasizes that these preventive screenings have an additive effect (AAP, 2017a). Conditions, such as lead poisoning or significant dyslipidemia, may cause irreversible damage during child development, and as such it is crucial to screen for these conditions. Due to the enormous variation in children and families, the AAP provides many recommendations in the form of a periodicity schedule; this schedule is meant for children "who are receiving competent parenting, have no manifestations of any important health problems, and are growing and developing in a satisfactory fashion." The AAP notes that developmental, psychosocial, or chronic issues may require additional counseling or treatment visits alongside the preventive care visits (AAP, 2017a).

The Bright Futures initiative was started in 1990 by the Maternal and Child Health Bureau to improve the health of children and prevent disease. The AAP partnered with Bright Futures, and these organizations have now issued joint guidelines and recommendations related to the screening of children and adolescents for common preventable and treatable disorders. The recommendations are age-related and aligned with the standard timing of medical visits for children (AAP, 2019, 2021a).

Clinical Utility and Validity

The AAP has noted a lack of strong evidence to support pediatric preventive screening for numerous conditions. However, the AAP has emphasized that "lack of evidence does not mean a lack of effectiveness" and has ensured that their recommendations have adequately assessed the benefit of screening against potential harm (AAP, 2017a).

The Centers for Disease Control and Prevention (CDC) estimates the number of newborn screenings to be 4 million a year in the United States (CDC, 2019a). The CDC performed a study assessing the number of conditions diagnosed because of screening newborns and identified approximately 12,500 diagnoses found due to the newborn screening programs equaling approximately 1 out of 4000 live births. Severe disorders are identified in approximately 5,000 newborns each year (CDC, 2019a). At the time of the study, the core screening panels consisted of 29 core conditions. The five most commonly diagnosed conditions were (in order): hearing loss, primary congenital hypothyroidism, cystic fibrosis, sickle cell disease, and medium-chain acyl-CoA dehydrogenase deficiency. The CDC estimated congenital hearing loss to occur in one to three live births out of 1000. Finally, the CDC estimated the cost of the newborn screening program to be about $30 per infant, or $120 million (CDC, 2012). The CDC has also developed a newborn screening and molecular biology branch (NSMBB), and a newborn screening quality assurance program (NSQAP), to assist in the development of analytical methods to measure substances in dried blood spots. Certified materials for newborn screening tests are also produced by this branch (CDC, 2019b).

IV. Guidelines and Recommendations

The American Academy of Pediatrics (AAP) and Bright Futures Recommendations for Preventive Pediatric Health Care

The American Academy of Pediatrics' (AAP) (through Bright Futures) recommendations include the following screenings. The Bright Futures/AAP Periodicity Schedule describes the screenings, assessments, physical examinations, procedures, and timing of anticipatory guidance recommended for each age-related visit. Below are the laboratory-related screening recommendations:

- Newborn blood and bilirubin
- Anemia risk assessment at 4 months, test at 12 months, and further risk assessments at each subsequent visit with appropriate action to follow, if positive
- Lead screening at 6, 9, 12, 18, and 24 months, and then once annually from 3-6 years, if indicated
- Tuberculosis screening at 1, 6, 12, and 24 months, and then annually thereafter starting at 3 years old, if indicated

- Dyslipidemia screening at 24 months and then every 2 years starting at 4 years old; AAP also recommends screening at least once between ages 9 and 11 and between 17 and 21. Annual risk assessments starting at age 12 up to age 16 are recommended, with appropriate action to follow, if positive.
- STI/HIV screening annually starting at 11 years old, with at least one HIV screening between 15 and 18 (AAP, 2017b, 2021b).

Many of these recommendations were based on the USPSTF's recommendations (AAP, 2017a).

The AAP has also released a policy statement on targeted testing for lead. The AAP recommends targeted testing for lead in immigrant, refugee, or internationally adopted children at time of arrival (AAP, 2016).

The Advisory Committee on Heritable Disorders in Newborns and Children recommendations are included in the Bright Futures' periodicity table. The committee recommends that every newborn screening program include a Recommended Uniform Screening Panel (RUSP) that screens for 35 core disorders and 26 secondary disorders (RUSP, 2020). Required screenings vary by state.

The core disorders are as follows: Propionic Acidemia, Methylmalonic Acidemia, (methylmalonyl-CoA mutase) Methylmalonic Acidemia, (Cobalamin disorders) Isovaleric Acidemia, 3-Methylcrotonyl-CoA Carboxylase Deficiency, 3-Hydroxy-3-Methyglutaric Aciduria, Holocarboxylase Synthase Deficiency, ß-Ketothiolase Deficiency, Glutaric Acidemia Type I, Carnitine Uptake Defect/Carnitine Transport Defect, Medium-chain Acyl-CoA Dehydrogenase Deficiency, Very Long-chain Acyl-CoA Dehydrogenase Deficiency, Long-chain L-3 Hydroxyacyl-CoA Dehydrogenase Deficiency, Trifunctional Protein Deficiency, Argininosuccinic Aciduria, Citrullinemia, Type I, Maple Syrup Urine Disease, Homocystinuria, Classic Phenylketonuria, Tyrosinemia Type I, Primary Congenital Hypothyroidism, Congenital adrenal hyperplasia, S,S Disease (Sickle Cell Anemia), S, βeta-Thalassemia, S,C Disease, Biotinidase Deficiency, Critical Congenital Heart Disease, Cystic Fibrosis, Classic Galactosemia Glycogen Storage Disease Type II (Pompe), Hearing Loss, Severe Combined Immunodeficiencies, Mucopolysaccharidosis Type 1, X-linked Adrenoleukodystrophy, Spinal Muscular Atrophy due to homozygous deletion of exon 7 in *SMN1*.

The secondary disorders are as follows: Methylmalonic acidemia with homocystinuria, Malonic acidemia, Isobutyrylglycinuria, 2-Methylbutyrylglycinuria, 3-Methylglutaconic aciduria, 2-Methyl-3-hydroxybutyric aciduria, Short-chain acyl-CoA dehydrogenase deficiency, Medium/short-chain L-3-hydroxyacylCoA dehydrogenase deficiency, Glutaric acidemia type II, Medium-chain ketoacyl-CoA thiolase deficiency, 2,4 Dienoyl-CoA reductase deficiency, Carnitine palmitoyltransferase type I deficiency, Carnitine palmitoyltransferase type II deficiency, Carnitine acylcarnitine translocase deficiency, Argininemia, Citrullinemia type II, Hypermethioninemia, Benign hyperphenylalaninemia, Biopterin defect in cofactor biosynthesis, Biopterin defect in cofactor regeneration, Tyrosinemia, type II, Tyrosinemia, type III, Various other hemoglobinopathies, Galactoepimerase deficiency, Galactokinase deficiency, T-cell related lymphocyte deficiencies (Children, 2020).

There is also another category set forth by the RUSP—conditions for which newborn screening is not indicated. These include conditions that do not have adequate testing or did not meet other criteria in the RUSP's review. These conditions are as follows: Krabbe disease, Pompe disease, Lysosomal storage

diseases, Creatine transport defect, Fabry disease, X-linked adrenoleukodystrophy, Hurler-Scheie disease, Biliary atresia, Smith-Lemli-Opitz syndrome, Congenital disorder of glycosylation type Ib, Fragile X syndrome, Duchenne and Becker muscular dystrophy, Congenital Cytomegalovirus infection, α1-Antitrypsin deficiency, Carbamylphosphate synthetase deficiency, Adenosine deaminase deficiency, Turner syndrome, Arginine: glycine amidinotransferase deficiency, Neuroblastoma, Diabetes mellitus, insulin dependent, Wilson disease, Guanidinoacetate methyltransferase deficiency, Ornithine transcarbamylase deficiency, Carnitine palmitoyltransferase IB deficiency (muscle), Familial hypercholesterolemia (heterozygote), Congenital Toxoplasmosis, Severe combined immunodeficiency, Neonatal hyperbilirubinemia (Kernicterus), Glucose 6-phosphate dehydrogenase deficiency (G6PD) (HHS, 2020b).

"Secondary" disorders refer to a class of conditions that are "part of the differential diagnosis of a core panel condition". The core disorders refer to conditions appropriate for newborn screening; they all "have specific and sensitive screening tests, a sufficiently well understood natural history, and available and efficacious treatments". Although states ultimately decide which conditions to screen for in their newborn screening programs, this list from the Department of Health and Human Services provides some standardization to those programs (HHS, 2018, 2020b).

American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE)

The 2017 AACE and ACE guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease Recommend the following for children:

- "In children at risk for FH (e.g., family history of premature cardiovascular disease or elevated cholesterol), screening should be at 3 years of age, between 9 and 11, and at age 18" (Jellinger et al., 2017).
- "Screen adolescents older than 16 years every 5 years or more frequently if they have ASCVD risk factors, have overweight or obesity, have other elements of the insulin resistance syndrome, or have a family history of premature ASCVD" (Jellinger et al., 2017)..

American Diabetes Association (ADA)

The ADA standards of Medical Care in Diabetes document state the following recommendations for children and adolescents' dyslipidemia testing:

- "Initial lipid testing should be performed when initial glycemic control has been achieved and age is ≥2 years. If initial LDL cholesterol is ≤100 mg/dL (2.6 mmol/L), subsequent testing should be performed at 9-11 years of age. Initial testing may be done with a nonfasting non-HDL cholesterol level with confirmatory testing with a fasting lipid panel" (ADA,2020).
- "If LDL cholesterol values are within the accepted risk level (<100 mg/Dl [2.6 MMOL/l]), a lipid profile repeated every 3 years is reasonable (ADA, 2020).

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends screening for Hepatitis B virus (HBV) in adolescents and adults who are at an increased risk for infection (Grade B) (USPSTF, 2020b). The USPSTF recommends screening for hepatitis C virus (HCV) infection in adults aged 18 to 79 years (Grade B) (USPSTF, 2020a).

In children and adolescents 20 years or younger, the USPSTF concludes that "the current evidence is insufficient to assess the balance of benefits and harms of screening for lipid disorders" (USPSTF, 2016).

The USPSTF recommends screening for syphilis in adolescents who have ever been sexually active and are at increased risk for syphilis infection. The USPSTF continues to recommend screening for syphilis in nonpregnant persons who are at increased risk for infection (USPSTF, 2022b).

The USPSTF recommends screening for chlamydia and gonorrhea in all sexually active women ages 24 and under (Grade B) (USPSTF, 2014, 2021).

The USPSTF has stated that there is insufficient evidence to assess the balance of benefits and harms of screening for elevated blood lead levels in asymptomatic children ages 1-5 years (Cantor et al., 2019).

The USPSTF recommends screening adolescents 15 and older for HIV infection. Adolescents under 15 but who are at increased risk should also be screened (Grade A) (Chou et al., 2019; USPSTF, 2019).

The USPSTF has deemed the current evidence insufficient for children ages 6-24 months to be screened for iron deficiency anemia (Siu, 2015).

The USPSTF recognized the importance of screening for hemoglobinopathies in newborns including sickle cell disease, but will not update this 2007 recommendation (USPSTF, 2007).

The USPSTF recognized the importance of screening for congenital hypothyroidism in newborns in 2008, but will not update this recommendation (USPSTF, 2008a).

The USPSTF recognized the importance of screening for phenylketonuria in newborns, but will not update this 2008 recommendation (USPSTF, 2008b).

The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for primary hypertension in asymptomatic children and adolescents to prevent subsequent cardiovascular disease (Moyer, 2013). A 2020 recommendation statement by the USPSTF confirmed that the current evidence is insufficient to assess the balance of benefits and harms of screening for high blood pressure in children and adolescents (in general) (USPSTF, 2022a).

Centers for Disease Control and Prevention (CDC)

The CDC acknowledges the Bright Future's and USPSTF recommendations for pediatric preventive screening, including HIV screening (CDC, 2018, 2020). On May 14, 2021, the CDC updated its blood lead reference value (BLRV) from 5 µg/dL to 3.5 µg/dL in response to a recommendation from the Lead Exposure and Prevention Advisory Committee (LEPAC). The BLRV is a metric used to identify children with blood lead levels that are higher than most (97.5th percentile) other children's levels (CDC, 2021).

With respect to the COVID-19 pandemic, the CDC recommends that "healthcare providers should identify children who have missed well-child visits and/or recommended vaccinations and contact them to schedule in-person appointments, with prioritization of infants, children age < 24 months and school-aged children. Developmental surveillance and early childhood screenings…should continue along with referrals for early intervention services and further evaluation if concerns are identified." Further, "newborn visits should be done in-person, even during the COVID-19 pandemic, to evaluate feeding and weight gain, check for dehydration and jaundice, [and] ensure all components of newborn screening were completed with appropriate confirmatory testing and follow-up…" (CDC, 2020b).

The American Academy of Family Physicians (AAFP)

The AAFP guidelines recommend various preventive services for children.

For newborns, the AAFP recommends congenital hypothyroidism screening, hearing loss screening, phenylketonuria screening, and sickle cell disease screening. This is closely aligned with USPSTF guidelines (Lin, 2015).

For sexually active adolescent females, the AAFP recommends gonorrhea and chlamydia infection screening (Lin, 2015). The AAFP supports the USPSTF recommendation for syphilis screening as listed above (AAFP, 2016).

For children and adolescents at high risk of infection, the AAFP recommends HIV and Hepatitis B screening (Lin, 2015).

To address and help rectify low-value care practices, Schefft et al. (2019) published on the inception of a series of "do and don't" recommendations in the delivery of healthcare for children and adolescents (Schefft et al., 2019). These recommendations include a suggestion for laboratory-based screening:

- "Don't routinely screen for hyperlipidemia in children and adolescents."

Turner (2018) confirms that the AAFP "generally adheres to USPSTF recommendations" and references several recommendations about screening from the USPSTF and AAP as listed below. The recommendations included below are only those that are within the scope of this medical policy (laboratory-based preventive screening tests):

Screening Recommendations for Children from Birth to 6 Years of Age:

- Dyslipidemia screening by a fasting lipid panel received a grade of "insufficient evidence" by the USPSTF. The AAP recommends "risk-based screening at 2, 4, and 6 years of age (SOR C)."
- Iron deficiency screening by complete blood count received a grade of "insufficient evidence" by the USPSTF. The AAP recommends "screen at 12 months; consider supplements for preterm or exclusively breastfed newborns (SOR C)."
- Lead poisoning screening by measuring lead levels. The USPSTF states that there is "insufficient evidence to recommend screening in children 1 to 5 years of age without increased risk (Grade I)." The AAP recommends "screen[ing] high-risk individuals 6 months to 6 years of age (SOR C)" (Turner, 2018).

National Lipid Association (NLA)

The guidelines list recommendations for "youth" (<20 years old), stating that "Measurement of Lp(a) may be reasonable with:

- Clinically suspected or genetically confirmed FH.
- Individuals with a family history of first-degree relatives with premature ASCVD (<55 y of age in men, 65 y of age in women)
- An unknown cause of ischemic stroke
- A parent or sibling found to have an elevated Lp(a)" (Wilson et al., 2022).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA has approved multiple tests for pediatric preventive screening.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Although the HHS has created the RUSP to provide some standardization for each state's newborn screening programs, the HHS emphasizes that the conditions screened in each program are ultimately decided by the states.

Public Health Service Act (PHS Act) (HHS)

As per the U.S. Department of Health and Human Services, Section 2713 of the PHS Act "generally requires group health plans and group and individual health insurance issuers that are not grandfathered health plans to provide coverage for recommended preventive services without cost sharing. A complete list of the current recommended preventive services is available at www.healthcare.gov/center/regulations/prevention.html" (HHS, 2020a).

National Association of State Boards of Education (NASBE)

The NASBE provides information about state mandates for school health screening (NASBE, 2022).

Please note that individual states may provide specific guidelines and recommendations for pediatric preventive screening.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
80061	Lipid panel - Lipid panel This panel must include the following: Cholesterol, serum, total (82465) Lipoprotein, direct measurement, high density cholesterol (HDL cholesterol) (83718) Triglycerides (84478)
82247	Bilirubin; total
82248	Bilirubin; direct
82465	Cholesterol, serum or whole blood, total
83020	Hemoglobin fractionation and quantitation; electrophoresis (eg, A2, S, C, and/or F)
83021	Hemoglobin fractionation and quantitation; chromatography (eg, A2, S, C, and/or F)
83655	Lead
83718	Lipoprotein, direct measurement; high density cholesterol (HDL cholesterol)
84439	Thyroxine; free
84443	Thyroid stimulating hormone (TSH)
84478	Triglycerides
85014	Blood count; hematocrit (Hct)
85018	Blood count; hemoglobin (Hgb)
86480	Tuberculosis test, cell mediated immunity antigen response measurement; gamma interferon
86580	Skin test; tuberculosis, intradermal
86701	Antibody; HIV-1
86702	Antibody; HIV-2
86850	Antibody screen, RBC, each serum technique
87391	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; HIV-2
87516	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis B, amplified probe technique
87517	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis B virus, quantification
87534	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, direct probe technique
87535	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, amplified probe technique, includes reverse transcription when performed
87555	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, direct probe technique
87556	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, amplified probe technique
88720	Bilirubin, total, transcutaneous
S3620	Newborn metabolic screening panel, includes test kit, postage and laboratory tests specified by the state for inclusion in this panel (e.g., galactose; hemoglobin,

Pediatric Preventive Screening, continued

	electrophoresis; hydroxyprogesterone, 17-D; phenylamine (PKU); and thyroxine, total)
0257U	Very long chain acyl-coenzyme A (CoA) dehydrogenase (VLCAD), leukocyte enzyme activity, whole blood Proprietary test: Very-Long Chain Acyl-CoA Dehydrogenase (VLCAD) Enzyme Activity Lab/Manufacturer: Children's Hospital Colorado Laboratory

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
4/29/22	Removed the word "children" from most criteria to focus requirements on actual ages. Also, added CPT code 0257U (Very long chain acyl-coenzyme A (CoA) dehydrogenase (VLCAD), leukocyte enzyme activity, whole blood Proprietary test: Very-Long Chain Acyl-CoA Dehydrogenase (VLCAD) Enzyme Activity Lab/Manufacturer: Children's Hospital Colorado Laboratory), which is a not-covered code with Select Health.
5/18/23	Removed coverage criteria #8b, which provided screening limitations for age groups 9 to 11 and 17 to 21.
10/18/23	The following changes were implemented: Coverage on screening for Hepatitis B screening (all ages), coverage on screening for chlamydia, gonorrhea, and/or syphilis infection (all ages), and coverage on HIV screening (all ages), were moved into other policies.

Human Immunodeficiency Virus (HIV)

Policy #: AHS – M2116	**Prior Policy Name & Number (as applicable):** AHS – M2116 – Plasma HIV-1 and HIV-2 RNA Quantification for HIV Infection AHS – M2116 – Plasma HIV-1 RNA Quantification for HIV-1 Infection
Implementation Date: 9/15/21	**Date of Last Revision:** 9/27/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Human immunodeficiency virus (HIV) is an RNA retrovirus that infects human immune cells, specifically CD4 cells, causing progressive deterioration of the immune system ultimately leading to acquired immune deficiency syndrome (AIDS) characterized by susceptibility to opportunistic infections and HIV-related cancers (CDC, 2014). HIV-1 is the dominant subtype of HIV infection, but another subtype, HIV-2, is a crucial subtype in certain areas of the world, such as Western Africa (Sax, 2019).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For individuals 11 to 65 years of age, initial screening for HIV infection **MEETS COVERAGE CRITERIA**.
2. For individuals 11 to 65 years of age, repeat screening for HIV infection (no less than 90 days after initial screening) **MEETS COVERAGE CRITERIA**.
3. HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** for any of the following situations:

a) Prior to initiating doravirine therapy (genotyping and phenotyping is required).

b) For individuals who have failed a course of antiviral therapy.

c) For individuals who have suboptimal viral load reduction.

d) For individuals who have been noncompliant with therapy.

e) To guide treatment decisions in individuals with acute or recent infection (within the last 6 months).

f) For antiretroviral naïve individuals entering treatment.

g) For all HIV-infected pregnant individuals in the following situations:

i) Before initiation of antiretroviral therapy

ii) For those with detectable HIV RNA levels

4. For treatment-experienced individuals on failing regimens who are thought to have multidrug resistance, HIV phenotyping **MEETS COVERAGE CRITERIA**.
5. When the risk of HIV infection is significant, and the initiation therapy is anticipated, a baseline HIV quantification **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) In an at-risk individual with persistence of borderline or equivocal serologic reactivity.

   b) In an at-risk individual with signs and symptoms of acute retroviral syndrome (characterized by fever, malaise, lymphadenopathy, and rash).
6. Plasma quantification of HIV-1 RNA or HIV-2 RNA (see Note 1) **MEETS COVERAGE CRITERIA** for any of the following situations:

   a) For monitoring disease progression in HIV-infected individuals

   b) For monitoring response to antiretroviral therapy.

   c) For infants younger than 18 months born to HIV-positive mothers (antibody tests may be confounded by maternal antibodies in this time frame).

   d) For predicting maternal-fetal transmission of HIV-1 or HIV-2.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

7. Routine use of combined genotyping and phenotyping **DOES NOT MEET COVERAGE CRITERIA**.
8. Drug susceptibility phenotype predictions using genotypic comparison to known genotypic/phenotypic database **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Because differences in absolute HIV copy number are known to occur using different assays, plasma HIV RNA levels should be measured by the same analytical method. A change in assay method may necessitate re-establishment of a baseline.

III. Scientific Background

Human immunodeficiency virus (HIV) targets the immune system, eventually hindering the body's ability to fight infections and diseases. If not treated, an HIV infection may lead to acquired immunodeficiency syndrome (AIDS) which is a condition caused by the virus. There are two main types of HIV: HIV-1 and HIV-2; both are genetically different. HIV-1 is more common and widespread than HIV-2.

HIV-1

Human immunodeficiency virus type 1 (HIV-1) RNA in blood can be measured using qualitative or quantitative techniques. Qualitative testing is used as a screening test to identify HIV-infected individuals whereas quantitative measurement of HIV-1 viral loads in the blood is used in management and monitoring of HIV-1 infected individuals. HIV-1 RNA levels may also be used to establish the diagnosis of HIV infection in specific situations where combination tests that detect HIV p24 antigen and HIV antibodies are not appropriate (neonatal or acute infection) (Caliendo, 2022).

Three primary real-time reverse transcriptase polymerase chain reaction (RT-PCR) commercial tests are commonly used to quantify HIV-1 RNA from plasma. These tests are more sensitive (detecting 20 to 40 copies/mL of HIV RNA), have a broader linear range (detecting virus to at least 10 million copies/mL), and pose a lower risk of carry over contamination than prior PCR assays. The tests are "COBAS TaqMan HIV-1 Test version 2" by Roche Diagnostics, "RealTime HIV-1" by Abbott Molecular, and "Aptima HIV-1 Quant Dx Assay" by Hologic (Caliendo, 2020). In 202, the Aptima assay recently received FDA approval to aid in diagnosis, in addition to its original use of quantitation (BusinessWire, 2020; FDA, 2020).

Sources of variability between assays include differences in technology platform, plasma input volume, and ability to detect HIV-1 subtypes. Monitoring of individual patients should be performed on the same technology platform to ensure appropriate interpretation of changes in viral load (Sollis et al., 2014). An important difference between assays is the gene target; with the increasing use of integrase inhibitors, monitoring for resistance mutations in the integrase gene is essential to ensure that the primer and probe binding sites are not impacted (Caliendo, 2022).

Overall, studies of real-time RT-PCR tests have shown high concordance, high correlation values, and good agreement among all assays (Mor et al., 2015). However, their manufacturers have reported that variation and error tend to increase at the lower limits of quantitation of the assays (Swenson et al., 2014). The high variability around the threshold of detectability of the viral load assays should be noted since many patients have viral loads in this range. Agreement between these assays was improved using a 200-copies/ml threshold (Swenson et al., 2014) consistent with the current HIV treatment guidelines' definition of virological failure (Saag et al., 2020).

Furthermore, changes in HIV-1 RNA levels must exceed at least 0.5 $\log_{10}$ or threefold in magnitude to represent biologically relevant changes in viral replication (Hughes et al., 1997; M. S. Saag et al., 1996).

Viral RNA levels can also transiently rise due to acute illness, herpes outbreak, or vaccination; however, values usually return to baseline within one month (Caliendo, 2022). CD4 cell counts are weakly correlated with viral RNA measurements. Viral RNA measurements, although, do not replace CD4 cell counts in the management of HIV-1-infected patients and should be used in parallel (Caliendo, 2022).

HIV-2

Human immunodeficiency virus type 1 (HIV-2) is another subtype of HIV. Compared to HIV-1, HIV-2 appears milder clinically; it is characterized by a longer asymptomatic stage, slower declines of CD4 cell counts, and lower levels of plasma viremia in chronically ill patients (Gottlieb, 2022b). However, these numerical thresholds are not as well-defined as those of HIV-1 as there is currently not as much data available for HIV-2. Further, although quantification of HIV-2 RNA viral load may be useful, it is not widely commercially available, as the few labs that offer HIV-2 testing only offer qualitative testing and not quantitative (Gottlieb, 2022b). This is particularly crucial as HIV-1 assays typically do not properly detect HIV-2 viral load (DHHS, 2022a). It is possible for commercially available HIV-1 diagnostic assays to cross-react with HIV-2, disrupting the results. A reactive HIV-1 Western Blot may not be indicative of a true HIV-1 infection. For example, a patient may have reactive HIV serology, but test negative on a confirmatory HIV-1 Western blot. This scenario may indicate an HIV-2 infection. Clinical manifestations of HIV-2 infection are generally similar to HIV-1 infection, but much remains to be discovered about the general course of HIV-2 infection (Gottlieb, 2022a).

Despite HIV-2's milder symptoms, certain clinical features may make an infection more difficult to manage; for example, HIV-2 is intrinsically resistant to non-nucleoside reverse transcriptase inhibitors, as well as enfuvirtide. Assessment of genotypic or phenotypic resistance is also unexplored, with no currently FDA-approved genotypic or phenotypic resistance assays available (DHHS, 2022a).

Although HIV-2 is endemic to West Africa (Senegal, Gambia, Guinea-Bissau, et al.) the epidemiological trends may be shifting; the CDC only reported 166 cases of HIV-2 from 1987 to 2009 but this may be underestimated as HIV-2 is often asymptomatic. There were 24 cases of HIV-2 identified in New York City between 2010 and 2020, with 25 additional probable cases. Additionally, as much as 5% of HIV cases are thought to be HIV-2 (Gottlieb, 2022b; Quinn, 2022).

Drug Resistance

HIV replicates rapidly; a replication cycle rate of approximately one to two days ensures that after a single year, the virus in an infected individual may be 200 to 300 generations removed from the initial infection-causing virus (Coffin & Swanstrom, 2013). This leads to great genetic diversity of each HIV infection in an individual. As an RNA retrovirus, HIV requires the use of a reverse transcriptase for replication purposes. A reverse transcriptase is an enzyme which generates complimentary DNA from an RNA template. This enzyme is error-prone with the overall single-step point mutation rate reaching about 3.4×10^{-5} mutations per base per replication cycle (Mansky & Temin, 1995), leading to approximately one genome in three containing a mutation after each round of replication (some of which confer drug resistance). This rate is comparable to other RNA viruses. This pace of replication, duration of infection, and size of the replicating population allows the retrovirus to evolve rapidly in response to selective influences (Coffin & Swanstrom, 2013).

Due to the high rate of mutation in HIV viruses, drug resistance mutations are common. Some drugs may be resisted by a single mutation—these drugs have a "low genetic barrier" to resistance. Such mutations are common enough to be termed "signature mutations," which are frequently associated with a specific drug resistance. For example, the K103N mutation commonly leads to resistance for efavirenz. Efavirenz is a standard retroviral medication used to treat and prevent HIV and AIDs. To combat this, medical professionals can now assess drug-resistant HIV variants using phenotypic testing and genotypic testing (Kozal, 2019a).

Genotypic assays detect the presence of specific drug-resistance mutations in several different genes (protease, reverse transcriptase, and integrase genes). For example, assays may test for resistance in nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs). The definition of a resistance conferring mutation is blurred, but generally includes one or more of the following conditions:

- The mutation confers phenotypic resistance when introduced into a drug-sensitive laboratory strain of HIV.
- The mutation is selected for during serial in vitro passage of the virus in the presence of a drug.
- The mutation is selected for during clinical therapy with that drug.
- The presence of the mutation in clinical isolates is associated with phenotypic resistance and virologic failure (Kozal, 2019b).

Interpretation of genotypic data may be done either by clinical expertise or through a database (in which the genotype is correlated with the phenotype). Phenotypic resistance assays measure the extent to which an antiretroviral drug inhibits viral replication. Phenotypic testing typically assesses the fold-change in susceptibility of a patient's virus and the treatment response, while also correlating the mutations present with the fold-change in susceptibility. Recombinant virus assays (RVAs) are used; protease, reverse transcriptase, or integrase gene sequences from circulating viruses are inserted into a reference strain of HIV, and this new HIV strain is measured by the phenotypic assay (Kozal, 2019b).

Several HIV genotypic assays are available. The ViroSeq HIV-1 Genotyping system by Abbott helps to detect HIV-1 genomic mutations that may lead to resistance to certain types of antiretroviral drugs (ThermoFisher, 2011). The ATCC® HIV-1 Drug Resistance Genotyping Kit has been developed by the American Type Culture Collection (ATCC), the Centers for Disease Control and Prevention (CDC) and Thermo Fischer Scientific; this is a real time- polymerase chain reaction (rt-PCR) assay which may help to identify and monitor HIV-1 drug resistance (ATCC, 2014).

The primary phenotypic assay is "PhenoSense" from LabCorp. The Human Immunodeficiency Virus 1 (HIV-1) PhenoSense GT® Plus Integrase (Monogram® Phenotype + Genotype) test by LabCorp measures HIV genotypic and phenotypic resistance from plasma samples (LabCorp, 2021).

Advantages of the genotype assays include lower cost and shorter turnaround time. However, interpretation of these assays is complicated by combinations of individual mutations that may have a differential effect on resistance that differs from the individual mutation alone (Kozal, 2019b). Mutation combinations are known to cause resistance to certain drugs, but increase susceptibility to others, impact viral fitness, and contribute to major pathways of resistance; additionally, the interactions of mutations

affecting various mechanisms can be difficult to predict. Over 20 rules-based genotypic interpretation systems (GIS) have been proposed (Fox et al., 2007; Kozal, 2019b).

Advantages of phenotypic assays include an ability to measure resistance more directly and examine the relative effect of multiple mutations on drug resistance. Limitations of the phenotypic assays include a longer turnaround time, greater expense, and biologic cut-offs above achievable drug levels. Phenotypic resistance assays may be helpful when evaluating HIV strains with known or suspected complex drug resistance mutation patterns as their actual resistance may not be accurately predicted by simply detecting the presence of multiple mutations (Kozal, 2019b). Both assays are limited by decreased sensitivity for low-level minority variants that comprise less than five to 20 percent of the virus population (Kozal, 2019b).

Analytical Validity

Rosemary et al. (2018) performed a comparison of two genotyping assays, ViroSeq and ATCC (manufactured by Thermo-Fisher Scientific) kit. A total of 183 samples with a viral load ≥1000 copies/mL were sequenced by ViroSeq and randomly selected (85 successfully genotyped, 98 unsuccessfully genotyped). The ATCC kit also genotyped 115 of the 183 samples, and out of the 98 unsuccessfully genotyped samples, the ATCC kit was able to genotype 42. Overall, 127 of the 183 samples were genotyped. The authors noted that the sequences of the genotyped samples were 98% identical and had "similar HIVDR profiles at individual patient level" (Rosemary et al., 2018).

Clinical Utility and Validity

Zhang et al. (2005) compared two phenotyping assays, Antivirogram and PhenoSense. Reverse transcriptase inhibitor susceptibility results were evaluated for 202 isolates from Antivirogram and 126 from PhenoSense. The authors found the median deviance for wild-type and mutant isolates to be lower for PhenoSense compared to Antivirogram, and PhenoSense was more likely to detect resistance to abacavir, didanosine, and stavudine when common drug resistance mutations were present (Zhang et al., 2005).

Hopkins et al. (2015) performed a study comparing the three main RT-PCR tests available, Aptima, COBAS TaqMan (CTM), and Abbott RealTime. The assays were evaluated based on plasma samples from 191 HIV positive patients as well as WHO International Standards (12-500 copies/mL). Aptima detected 141/191 (74%) of the HIV samples, CTM detected 145/191 (76%), and Abbott RealTime detected 119/191 (62%). The authors noted that precision decreased as the viral load got closer to the lower limit of quantification of 50 copies/mL (Hopkins et al., 2015).

Sempa et al. (2016) evaluated the utility of HIV-1 viral load as a prognostic indicator. A total of 489 patients were evaluated, and the viral load curves were evaluated on a linear scale and a logarithmic scale. The authors found that the viral load curve on the logarithmic scale was a statistically significant predictor of mortality, noting that each log10 increase in viral load corresponded to a 1.63 times higher risk of mortality. However, the authors stress that the choice of variables and statistical model influences the predictive power of this metric (Sempa et al., 2016).

Shen et al. (2016) assessed the ability to predict phenotypic drug resistance from genotypic data. The authors used two machine learning algorithms to predict drug resistance to HIV protease inhibitors and

reverse transcriptase inhibitors as well as the severity of that resistance from a query sequence. The accuracy of these classifications was found to be >0.973 for eight PR inhibitors and 0.986 for ten RT inhibitors and the r^2 was 0.772–0.953 for the PR cohort and 0.773–0.995 for the RT cohort. The algorithms' results were verified by "five-fold cross validation" on the genotype-phenotype datasets (Shen et al., 2016).

Lindman et al. (2019) investigated the test performance of the Bio-Rad Geenius HIV-1/2 confirmatory assay against INNO-LIA HIV 1/2 Score and ImmunoComb HIV 1/2 BiSpot. The Geenius test is purported to differentiate between HIV-1 and HIV-2 infections. 131 samples from ART naïve HIV infected patients in Guinea-Bissau were evaluated. The Geenius test identified 62 samples as "HIV-1 reactive", 37 as "HIV-2 reactive" and 32 as "HIV-1/2 dually reactive". INNO-LIA identified 63 as HIV-1 reactive, 36 as HIV-2 reactive, and 32 as HIV-1/2 dually reactive. The agreement between Geenius compared to INNO-LIA and Immunocomb was 92.4% and 84% respectively.

Avram et al. (2019) compared the cost-effectiveness of measuring viral load to guide delivery in HIV-positive women and compared it to routine cesarian delivery. A theoretical cohort of 1275 women was used, and the authors produced a decision-analytic model to compare the two techniques. The average cost of a point-of-care HIV RNA viral load test was placed at $15.22. The authors also assumed that each woman in the cohort would deliver two children. The authors defined the primary outcomes as "mother-to-child transmission, delivery mode, cesarean delivery-related complications, cost, and quality-adjusted life years", and the cost-effectiveness threshold was $100,000/quality-adjusted life year. The authors found that measuring viral load resulted in more HIV-infected neonates than routine cesarian delivery for all due to "viral exposure during more frequent vaginal births in this strategy". The authors found an increased cost of $3,883,371 and decreased quality-adjusted life years of 63 in the measurement strategy compared to the routine cesarian delivery strategy. At $100,000/quality-adjusted life year, measuring viral load was found to be cost-effective only "when the vertical transmission rate in women with high viral load below 0.68%" (compared to a baseline of 16.8%) and "when the odds ratio of vertical transmission with routine cesarean delivery for all compared with vaginal delivery was above 0.885" (compared to a baseline of 0.3). The authors concluded that "for HIV-infected pregnant women without prenatal care, quantifying viral load to guide mode of delivery using a point-of-care test resulted in increased costs and decreased effectiveness when compared with routine cesarean delivery for all, even after including downstream complications of cesarean delivery" Avram et al., 2019).

Raymond et al. (2020) evaluated the performance of the Vela Dx Sentosa next-generation sequencing (NGS) system for HIV-1 DNA genotypic resistance. 40 DNA samples were analyzed with Vela Dx Sentosa assay and the results were compared with Sanger sequencing. The Vela Dx Sentosa assay was 100% successful in amplifying and sequencing the protease and reverse transcriptase, and 86% successful in amplifying integrase sequences when the HIV DNA load was greater than 2.5 log copies/million cells. The Sentosa and Sanger sequencing were concordant for predicting protease-reverse transcriptase resistance in 20% of the 14/18 samples which were successfully sequenced. Sentosa was able to predict a higher level of resistance in three of the samples. The Vela Dx Sentosa predicted the prevalence of drug resistance to protease inhibitors (7%), nucleoside reverse transcriptase inhibitor (59%), nonnucleoside reverse transcriptase inhibitor (31%), and integrase inhibitors (20%). Overall, the authors conclude that the Vela Dx Sentosa assay can accurately predict HIV DNA drug resistance (Raymond et al., 2020).

Fogel et al. (2020) also analyzed the ability of next-generation sequencing methods to analyze HIV drug resistance. In this case, 145 plasma samples were analyzed using the ViroSeq HIV-1 Genotyping System and the veSEQ-HIV assay. Results were compared with the Abbott RealTime Viral Load assay. 142 HIV protease and reverse transcriptase sequences and 138 integrase sequences were obtained with ViroSeq. On the other hand, veSEQ-HIV detected 70.4% of the samples with protease, reverse transcriptase, and integrase sequences. Drug resistance mutations were detected in 33 ViroSeq samples and 42 veSEQ-HIV samples. Overall, veSEQ-HIV predicted more drug resistance mutations and worked better for larger viral loads. Results from veSEQ-HIV strongly correlated with the results from Abbott RealTime Viral Load assay. The authors conclude that the veSEQ-HIV assay provided results for most samples with higher viral loads, was accurate for predicting drug resistance mutations, but detected mutations at lower levels compared with the ViroSeq assay (Fogel et al., 2020).

Pröll et al. (2022) investigated whether NGS from proviral DNA and RNA could be an alternative to using plasma viral RNA as the material of choice for genotypic resistance testing at the start of ART and virologic failure for patients with low viremia. When taking samples from 36 patients, with varying viral loads of 96 to 390,000 copies/mL, the researchers found 2476 variants/drug resistance mutations by SS, while 2892 variants were found by NGS. Researchers stated, "An average of 822/1008 variants were identified in plasma viral RNA by Sanger or NGS sequencing, 834/956 in cellular viral RNA, and 820/928 in cellular viral DNA." This demonstrates that cellular RNA and cellular viral DNA could serve as viable substitutes when testing for variant detection and genotypic resistance among patients with HIV and low viremia (Pröll et al., 2022).

Ehret et al. (2022) tested the performance of the "Xpert® HIV-1 Viral Load (VL) XC" HIV-RNA quantitative assay made by Cepheid. This assay has been redesigned to use a dual-target approach. The authors tested 533 fresh and frozen samples from HIV-1 positive patients on the Abbott HIV-assay and the Xpert XC assay. "The Xpert XC assay yielded valid results in 98.5% (N = 528/536) of cases." The authors conclude that "The Xpert XC assay showed excellent correlation with the Abbott assays for all tested HIV-1 subtypes" (Ehret et al., 2022).

IV. Guidelines and Recommendations

Department of Health and Human Services (DHHS)

The Department of Health and Human Services (DHHS) Panel on Antiretroviral Guidelines for Adults and Adolescents updated the guidelines on use of antiretroviral drugs in 2022. The panel states "viral load is the most important indicator of initial and sustained response to ART and should be measured in all HIV-infected patients at entry into care (AI), at initiation of therapy (AI), and on a regular basis thereafter. Pre-treatment viral load level is also an important factor in the selection of an initial ARV regimen because several currently approved ARV drugs or regimens have been associated with poorer responses in patients with high baseline viral load."

The panel's recommendations on the frequency of viral load monitoring are summarized below (DHHS, 2022a):

- "After initiation of ART: Plasma viral load should be measured before initiation of ART and within 4 to 8 weeks after treatment initiation (AIII). The purpose of the measurements is to confirm an adequate

virologic response to ART, indicating appropriate regimen selection and patient adherence to therapy. Repeat viral load measurement should be performed at 4- to 8-week intervals until the level falls below the assay's limit of detection (BIII)."

- "In patients with viral suppression, with ART modification because of drug toxicity or for regimen simplification: Viral load measurement should be performed within 4 to 8 weeks after changing therapy (AIII). The purpose of viral load monitoring at this point is to confirm the effectiveness of the new regimen."

- "In patients on a stable, suppressive ARV regimen: Viral load measurement should be repeated every 3 to 4 months (AIII) or as clinically indicated to confirm continuous viral suppression. Clinicians may extend the interval to 6 months for adherent patients whose viral load has been suppressed for more than a year, whose clinical and immunologic status is stable, and who are not at risk for inadequate adherence (AIII)."

- "In patients with virologic failure who require a change in ARV regimen: Plasma viral load should be measured before ART change and within 4 to 8 weeks after treatment modification (AIII). The purpose of the measurements is to confirm an adequate virologic response to the new regimen. Repeat viral load measurement should be performed at 4- to 8-week intervals until the level falls below the assay's limit of detection (BIII). If viral suppression is not possible, repeat viral load measurement every 3 months or more frequently if indicated (AIII)."

- "In patients with suboptimal response: The frequency of viral load monitoring will depend on clinical circumstances, such as adherence and availability of further treatment options. In addition to viral load monitoring several other factors, such as patient adherence to prescribed medications, suboptimal drug exposure, or drug interactions, should be assessed. Patients who fail to achieve viral suppression should undergo resistance testing to aid in the selection of an alternative ARV regimen."

The guideline also comments on HIV-2. Although the optimal treatment strategy has not been defined, the guideline does recommend that quantitative plasma HIV-2 RNA viral load testing should be performed before initiating ART (AIII). HIV-2 RNA should also be used to assess treatment response. The guideline also notes that the "Geenius HIV 1/2 Supplemental Assay (Bio-Rad Laboratories)" is FDA-approved to differentiate HIV-1 infection from HIV-2 infection (DHHS, 2022a).

The CDC refers to the above guidelines on their website (CDC, 2021).

In an updated review in 2022, the DHHS also strongly recommended (AIII) hat "A blood sample for genotypic testing should be sent to the laboratory before initiation of ART." Moreover, "Pregnancy testing should be performed in persons of childbearing potential before initiation of ART."

The DHHS propounds further, stating the following:

- "Combination immunoassays that detect HIV-1 and HIV-2 antibodies and HIV p24 antigen (Ag/Ab assays) are part of the recommended initial laboratory HIV testing algorithm, primarily due to their enhanced ability to detect acute HIV infection. Specimens that are reactive on an initial Ag/Ab assay should be tested with an immunoassay that differentiates HIV-1 from HIV-2 antibodies. Specimens that are reactive on the initial assay and have either negative or

indeterminate antibody differentiation test result should be tested for quantitative or qualitative HIV RNA; an undetectable HIV RNA test result indicates that the original Ag/Ab test result was a false positive. Detection of HIV RNA in this setting indicates that acute HIV infection is highly likely."

- "HIV infection should be confirmed by repeat quantitative HIV RNA testing or subsequent testing to document HIV antibody seroconversion."
- "The proposed threshold of <3,000 copies/mL is based on historical data that used laboratory methods that are now considered obsolete.[18] These older viral load assays demonstrated false-positive cases of acute HIV infection at HIV RNA levels of <3,000 copies/mL. However, improvements in plasma viral load methodology suggest that any positive result on a quantitative plasma HIV RNA test in the setting of a negative or indeterminate antibody test result is highly consistent with acute HIV infection, including at HIV RNA levels of <3,000 copies/mL. HIV RNA levels in acute infection are generally very high (e.g., >100,000 copies/mL);[1, 2, 4] however, levels may be <3,000 copies/mL in the earliest weeks following infection as viral load continues to rise. Therefore, when a low-positive quantitative HIV RNA test result is present at this level, the HIV RNA test should be repeated on a new blood specimen to confirm the diagnosis. Repeated false-positive HIV RNA test results are unlikely" (DHHS, 2022a).

As persons who acquire HIV while taking pre-exposure prophylaxis (PrEP) may present ambiguous HIV test results, the DHHS proposes that:

- "A positive HIV Ag/Ab test result or a positive HIV RNA test result in the setting of a negative HIV antibody test result should prompt immediate confirmation of HIV diagnosis. It is important to collect a new blood specimen to verify the HIV diagnosis before initiating ART."
- "In people with HIV RNA level ≥200 copies/mL who are taking PrEP, immediate initiation of an effective HIV treatment regimen is recommended while awaiting confirmation of HIV diagnosis (AIII)."
- "In people taking PrEP who have a negative HIV antibody test result and a very low-positive quantitative HIV RNA test result (≥200 copies/mL) a confirmatory HIV antibody test and repeat quantitative plasma HIV RNA test should be performed, and results should be available before initiating ART."
- "In rare cases, particularly when PrEP is transitioned to an antiretroviral (ARV) regimen and HIV RNA and antibody diagnostic testing are inconclusive, HIV DNA testing may be of value" (DHHS, 2022a).

The Department of Health and Human Services (DHHS, 2022a, 2022b, 2022c) updated their guidelines for using drug resistance assays in HIV infections. The guidelines recommend HIV genotyping or phenotyping in the following situations among pregnant individuals and reducing perinatal HIV transmission in the US:

- "General Principles Regarding Use of Antiretroviral Drugs During Pregnancy:
  - Antiretroviral (ARV) drug-resistance genotype evaluations or assays should be performed before starting ARV drug regimens in people who are ARV-naive (AII) or ARV-experienced (AIII) and before modifying ARV drug regimens (AII) in people whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL).
  - In pregnant people who are not already receiving ART, ART should be initiated before results of drug resistance testing are available because earlier viral suppression has been associated

with lower risk of transmission. When ART is initiated before results are available, the regimen should be modified, if necessary, based on resistance assay results (AII)."

- "Pregnant People with HIV Who Have Never Received Antiretroviral Drugs (Antiretroviral Naive)
  - The results of ARV drug-resistance studies should guide the selection of ARV regimens in people whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL) (see Antiretroviral Drug Resistance and Resistance Testing in Pregnancy) (AII). However, ART initiation should not be delayed while awaiting results of resistance testing. When ART is initiated before the results of the drug resistance assays are available, the ARV regimen should be modified, if necessary, based on the resistance assay results (AII)."
- "Pregnant People with HIV Who Are Currently Receiving Antiretroviral Therapy
  - ARV drug-resistance testing should be performed to assist the selection of active drugs when changing ARV regimens in pregnant people who are experiencing virologic failure on ART and who have HIV RNA levels >500 copies/mL to 1,000 copies/mL (AII). In individuals who have HIV RNA levels >500 copies/mL but <1,000 copies/mL, testing may be unsuccessful but still should be considered (BII)."
- "Pregnant People with HIV Who Have Previously Received Antiretroviral Medications but Are Not Currently Receiving Any Antiretroviral Medications
  - If HIV RNA is above the threshold for standard genotypic drug resistance testing (i.e., >500 to 1,000 copies/mL), ARV drug-resistance testing should be performed prior to starting an ARV drug regiment (AIII)
  - ART should be initiated prior to receiving results of current ARV resistance assays. ART should be modified based on the results of the resistance assay, if necessary (AII)."
- "Monitoring during Pregnancy
  - HIV drug-resistance testing (genotypic testing and, if indicated, phenotypic testing) should be performed during pregnancy in those whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL) before –
    - Initiating ART in antiretroviral (ARV)-naive pregnant people who have not been previously tested for ARV drug resistance (AII);
    - Initiating ART in ARV-experienced pregnant people (including those who have received preexposure prophylaxis) (AIII); or
    - Modifying ARV regimens for people with HIV who become pregnant while receiving ARV drugs or people who have suboptimal virologic response to ARV drugs that were started during pregnancy (AII).
  - ART should be initiated in pregnant patients prior to receiving the results of ARV-resistance tests. ART should be modified, if necessary, based on the results of resistance testing (AII)."
- "Antiretroviral Drug Resistance and Resistance Testing in Pregnancy
  - HIV drug-resistance testing (genotypic and, if indicated, phenotypic) should be performed in persons living with HIV whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 to 1,000 copies/mL) before
    - Initiating ART in ARV-naive pregnant women who have not been previously tested for ARV resistance (AII),
    - initiating ART in ARV-experienced pregnant women (including those who have received pre-exposure prophylaxis) (AIII), *or*

        - modifying ART regimens for those who are newly pregnant and receiving ARV drugs or who have suboptimal virologic response to the ARV drugs during pregnancy (AII).
    - Phenotypic resistance testing is indicated for treatment-experienced persons on failing regimens who are thought to have multidrug resistance (BIII).
    - ART should be initiated in pregnant persons before receiving results of ARV-resistance testing; ART should be modified, if necessary, based on the results of resistance assays (AII).
    - If the use of an integrase strand transfer inhibitor (INSTI) is being considered and INSTI resistance is a concern, providers should supplement standard resistance testing with a specific INSTI genotypic resistance assay (AIII). INSTI resistance may be a concern if-
        - a patient received prior treatment that included an INSTI, *or*
        - a patient has a history with a sexual partner on INSTI therapy who was not virologically suppressed or with unknown viral load
        - documented zidovudine (ZDV) resistance does not affect the indications for use of intrapartum intravenous ZDV (BIII)." (DHHS, 2022c).

Among adults and adolescents living with HIV, the DHHS recommends the following for drug resistance testing:

- "For initial treatment:
    - HIV drug-resistance testing is recommended at entry into care for persons with HIV to guide selection of the initial antiretroviral therapy (ART) regimen (AII). If therapy is deferred, repeat testing may be considered at the time of ART initiation (CIII)
    - Genotypic, rather than phenotypic, testing is the preferred resistance testing to guide therapy in antiretroviral (ARV)-naive patients (AIII)
    - In persons with acute or recent (early) HIV infection, in pregnant people with HIV, or in people who will initiate ART on the day of or soon after HIV diagnosis, ART initiation should not be delayed while awaiting resistance testing results; the regimen can be modified once results are reported (AIII)
    - Standard genotypic drug-resistance testing in ARV-naive persons involves testing for mutations in the reverse transcriptase and protease genes. If transmitted integrase strand transfer inhibitor (INSTI) resistance is suspected or if the person has used long-acting cabotegravir (CAB-LA) as pre-exposure prophylaxis (PrEP) in the past, providers should ensure that genotypic resistance testing also includes the integrase gene (AIII).
- For Antiretroviral Therapy-Experienced Persons:
    - HIV drug-resistance testing should be performed to assist the selection of active drugs when changing ART regimens in the following patients:
        - People with virologic failure and HIV-RNA levels >200 copies/mL (AI for >1,000 copies/mL, AIII for 501–1,000 copies/mL, CIII for confirmed HIV RNA 201–500 copies/mL). For people with confirmed HIV-RNA levels >200 copies/mL but >500 copies/mL, drug-resistance testing may be unsuccessful but should still be considered.
        - Persons with suboptimal viral load reduction (AII)
    - Reverse transcriptase and protease genotypic resistance testing should be performed on everyone with virologic failure; integrase resistance testing (which may need to be ordered separately) should be performed on individuals experiencing virologic failure while receiving an INSTI-based regimen (AII).

- For persons taking a non–long-acting ARV regimen, drug-resistance testing in the setting of virologic failure should be performed while the person is still taking their ARV regimen or, if that is not possible, within 4 weeks after discontinuing their ARV regimen (AII). If more than 4 weeks have elapsed since the non–long-acting agents were discontinued, resistance testing may still provide useful information to guide therapy; however, it is important to recognize that previously-selected resistance mutations can be missed due to lack of drug-selective pressure (CIII).
- Given the long half-lives of the long-acting injectable ARV drugs, resistance testing (including testing for resistance to INSTIs) should be performed in all persons who have experienced virologic failure on a regimen of long-acting CAB and rilpivirine or acquired HIV after receiving CAB-LA as PrEP, regardless of the amount of time since drug discontinuation (AIII).
- Genotypic testing is preferred over phenotypic-resistance testing to guide therapy in people with suboptimal virologic response or virologic failure while on first- or second-line regimens and in people in whom resistance mutation patterns are known or not expected to be complex (AII).
- The addition of phenotypic- to genotypic resistance testing is recommended for people with known or suspected complex drug-resistance mutation patterns (BIII).
- All prior and current drug-resistance test results, when available, should be reviewed and considered when constructing a new regimen for a patient (AIII)" (DHHS, 2022a).

In terms of the usage of drug-resistance assays among adolescents and adults with HIV, the DHHS recommends the following:

- "In acute or recent (early) HIV infection: Drug-resistance testing is recommended (AII). A genotypic assay is generally preferred (AIII). Treatment should not be delayed while awaiting results of resistance testing (AIII).
  - If ART is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AIII)."
- "In ART-naive patients with chronic HIV: Drug-resistance testing is recommended at entry into HIV care to guide selection of initial ART (AII). A genotypic assay is generally preferred."
  - For pregnant persons, or if ART will be initiated on the day of or soon after HIV diagnosis, treatment can be initiated prior to receiving resistance testing results.
  - If an INSTI is considered for an ART-naïve patient and/or transmitted INSTI resistance is a concern, providers should supplement standard resistance testing with a specific INSTI genotypic resistance assay, which may need to be ordered separately (AIII).
  - If therapy is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AIII)."
- "In patients with virologic failure: Drug-resistance testing is recommended in patients on combination ART with HIV-RNA levels >200 copies/mL (AI for >1,000 copies/mL, AIII for 501–1,000 copies/mL) and a confirmed HIV RNA 201–500 copies/mL (CIII). In patients with confirmed HIV-RNA levels between 201–500 copies/mL, testing may not be successful but should still be considered."
  - "Resistance testing should be done while the patient is taking ART or, if that is not possible, within 4 weeks after discontinuation of non–long-acting ARV drugs (AII). If >4 weeks have

elapsed, resistance testing may still be useful to guide therapy; however, previously-selected mutations can be missed due to lack of drug-selective pressure (CIII)."
    - "A standard genotypic resistance assay is generally preferred for patients experiencing virologic failure on their first or second ARV regimens and for those with expected noncomplex resistance patterns (AII)"
    - "All prior and current drug-resistance testing results should be reviewed and considered when designing a new ARV"
    - "When virologic failure occurs in a patient on an INSTI-based regimen or in a patient with a history of INSTI use, genotypic testing for INSTI resistance should be performed to determine whether to include drugs from this class in subsequent regimens (AII)."
    - "Adding phenotypic testing to genotypic testing is generally preferred in patients with known or suspected complex drug-resistance patterns (BIII)"
- "In patients with suboptimal suppression of viral load: Drug-resistance testing is recommended in patients with suboptimal viral load suppression after initiation of ART (AII)"
- "In Pregnant People with HIV: Genotypic resistance testing is recommended for all pregnant people before initiation of ART (AIII) and for those entering pregnancy with detectable HIV-RNA levels while on therapy (AI)."
- "In Patients with Undetectable Viral Load or Low-Level Viremia Who Are Planning to Change Their ARV Regimen HIV-1: proviral DNA resistance assays may be useful in patients with HIV RNA below the limit of detection or with low-level viremia, where a HIV-RNA genotypic assay is unlikely to be successful (CIII)" (DHHS, 2022a).

The DHHS also added guidelines on genotypic and phenotypic testing for pediatric HIV infection:

- "Antiretroviral (ARV) drug-resistance testing is recommended at the time of HIV diagnosis, before initiation of therapy, in all ART naive patients, and before switching regimens in patients with treatment failure (AII). Genotypic resistance testing is preferred for this purpose (AIII)."

"Phenotypic resistance testing should be considered (usually in addition to genotypic resistance testing) for patients with known or suspected complex drug resistance mutation patterns, which generally arise after a patient has experienced virologic failure on multiple ARV regimens (CIII)" (DHHS, 2022b)

International Antiviral Society

The International Antiviral Society published a 2022 update titled "Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults." The guideline also recommends laboratory testing to "characterize" the HIV stage prior to starting antiretroviral testing (ART); this is done by assessing HIV RNA level. (Gandhi, et al. 2021)

The guideline also remarks on the frequency of testing during ART. Their recommendations are as follows:

- "Within 6 weeks of starting ART, assessment of treatment adherence and tolerability is recommended, along with the measurement of HIV RNA level."

- "If the HIV RNA level has not declined by 2 log10 copies/mL within 12 weeks of therapy and adherence appears to be sufficient, then a genotype based on the patient's regimen is

recommended

- "If the patient remains virally suppressed, clinically stable, and adherent to medications, then HIV RNA levels should be monitored every 3 months until virally suppressed for at least 1 year. Afterward, the frequency of viral monitoring can be changed to every 6 months."

- "If HIV RNA is greater than 200 copies/mL on 2 consecutive measurements, then HIV RT-pro genotype and InSTI [in integrase strand transfer inhibitor] genotype (if the patient was receiving an InSTI) testing are recommended."

- "For patients with intermittent or persistent low-level viremia between 50 and 200 copies/mL, assessments for ART adherence, tolerability, and toxic effects are recommended, but changing ART regimens is not recommended unless ART toxicity or intolerability are identified" (Gandhi et al., 2022).

On resistance test, the 2022 update notes that, "in persons diagnosed with HIV while receiving TXF-based PrEP, resistance testing should be performed but initiation of ART need not be delayed while awaiting genotype results." The panel further recommends:

- "Unless there is documented or suspected history of treatment failure, proviral resistance testing is not required prior to switching to 2-drug therapy, even if there is no available pretreatment resistance test result."
- "For patients who have maintained viral suppression, switching from long-acting injectable cabotegravir plus rilpivirine back to daily oral therapy can be done without the need for proviral DNA resistance testing."
- "If virologic failure is confirmed, genotype resistance testing should be performed, preferably while patients are taking the failing therapy. Resistance testing is still recommended even if a regimen has been discontinued or a person acknowledges poor medication adherence" (Gandhi et al., 2022).

Infection Diseases Society of America (IDSA) (Thompson et al., 2020)

The IDSA recommends that "A quantitative HIV RNA (viral load) level should be obtained upon initiation of care (strong recommendation, high quality evidence)" (Thompson et al., 2020).

IDSA recommends rechecking HIV RNA after 2-4 weeks of initiating antiretroviral therapy (ART) (and no later than 8 weeks). From there, IDSA recommends "checking HIV RNA every 4-8 weeks until suppression is achieved". The IDSA also notes that viral load "should" be monitored every 3-4 months to "confirm maintenance of suppression below the limit of assay detection", 6 months for "adherent patients whose viral load has been suppressed for more than 2 years and whose clinical and immunologic status is stable", and more frequently after initiation or change in ART (IDSA recommends within 2-4 weeks of initiation or change but not more than 8 weeks) (Thompson et al., 2020).

Overall, IDSA lists two primary uses for viral load testing; to establish baseline and to monitor viral suppression (Thompson et al., 2020).

American College of Obstetricians and Gynecologists (ACOG)

In 2014, ACOG released "Committee on Gnecologic Practice: Routine human immunodeciency virus screening," which they reaffirdmed in 2020. Regarding routine human immunodeficiency screening, "The American College of Obstetricians and Gynecologists (the College) recommends routine HIV screening for females aged 13–64 years and older women with risk factors. Screening after age 64 years is indicated if there is ongoing risk of HIV infection, as indicated by risk assessment (eg, new sexual partners)" (ACOG, 2014).

The College also expatiates upon repeat testing, entrusting obstetrician–gynecologists to annually review patients' risk factors for HIV and assess their needs, and recommends that "HIV testing should be offered at least annually to women who

- are injection drug users
- are sex partners of injection-drug users
- exchange sex for money or drugs
- are sex partners of HIV-infected persons
- have had sex with men who have sex with men since the most recent HIV test
- have had more than one sex partner since their most recent HIV test

The opportunity for repeat testing should be made available to all women even in the absence of identified risk factors. Repeat screening after age 64 years is indicated if there is ongoing risk of HIV infection, as indicated by an individualized risk assessment. Obstetrician–gynecologists also should encourage women and their prospective sex partners to be tested before initiating a new sexual relationship. The benefits of periodic retesting should be discussed with patients and provided if requested, regardless of risk factors. Patients may be concerned about their status and do not know about or want to disclose risk-taking behavior to their health care providers" (ACOG, 2014).

In their 2018 committee opinion "Labor and Delivery Management of Women With Human Immunodeficiency Virus Infection," ACOG notes that current and ongoing research has shown that "treatment of HIV-infected pregnant women with combined antiretroviral therapy can achieve a 1–2% or lower risk of mother-to-child transmission if maternal viral loads of 1,000 copies/mL or less can be sustained, independent of the route of delivery or duration of ruptured membranes before delivery." ACOG further observes that "the risk of mother-to-child transmission in HIV-infected women with high viral loads can be reduced by performing cesarean deliveries before the onset of labor and before rupture of membranes [cesarean delivery in this document [the ACOG guideline]), in conjunction with the use of peripartum maternal antiretroviral therapy."

ACOG recommends offering a "scheduled prelabor cesarean delivery at 38 0/7 weeks of gestation to reduce the risk of mother-to-child transmission" if an HIV-positive pregnant woman is found to have a viral load of over 1000 copies/mL at or near delivery, independent of antepartum antiretroviral therapy. This recommendation also applies to patients whose viral load is unknown (ACOG, 2018).

Society for Maternal-Fetal Medicine (SMFM)

The SMFM published a "checklist for pregnancy management in persons with HIV". Although these

checklists are not definitive, they are intended to "help ensure that all relevant elements are considered for every person with HIV during prepregnancy, antepartum, intrapartum, and postpartum periods." During the third trimester, the checklist calls for viral load to be assessed at 34-36 weeks for delivery planning (and to assess adherence and viral resistance if viral load is not suppressed). Further, if the viral load is found to be ≥1000 copies/mL at 37-38 weeks, a caesarean delivery should be scheduled for 38 weeks (Gibson & Toner, 2020).

British HIV Association (BHIVA, 2019)

The British HIV Association (BHIVA) makes several recommendations regarding assessment of viral load during the routine investigation and/or maintenance of HIV-1 positive adults. Relevant recommendations are as follows:

- "We recommend that an HIV viral load should be performed at the first visit following serological diagnosis (1A).
- We recommend that undetectable viral load result whilst not on treatment needs repeating, review of serology to exclude HIV-2 and measurement on a different viral load assay (1D).
- We recommend a repeat HIV viral load in all new transfers prior to repeat prescriptions if it is not possible to confirm a recent viral load from the previous clinic (1A).
- We recommend that viral load measurements be taken at 1, 3 and 6 months after starting ART (1B).
- We recommend that additional viral load measurements are taken between 2 and 5 months after starting ART if viral load has not decreased at least 10-fold after 1 month of ART or there are concerns about the patient's adherence to therapy (1D).
- We recommend that viral load testing should be performed routinely every 6 months (1A) and might be at intervals of up to 12 months for patients established on ART that includes a PI (GPP) [general practice point].
- We recommend that viral load rebound to above 50 copies/mL should be confirmed by testing a subsequent sample (2A). Repeat testing of the same sample is not recommended.
- For patients stable on ART we recommend that:
  - Frequent (3–4 monthly) viral load follow-ups of individuals with stable unsuppressed (<200 copies/mL) viral loads if they are managed as low-level viraemic patients according to the BHIVA treatment guidelines (1D).
  - CSF HIV viral load measurement should be considered to exclude compartmentalisation (1C)" (BHIVA, 2019).

The BHIVA released guidelines for the management of HIV-2 (BHIVA, 2021). For the diagnosis of HIV-2, the BHIV recommends:

For the diagnosis of chronic HIV-2:

- "An initial diagnosis of chronic HIV-2 infection should be made using a total of three CE-marked serology tests (i.e. tests conform to EU health and safety requirements) performed in an ISO 15189-accredited laboratory. There must be reactivity in two CE-marked fourth-generation tests for HIV-1 and HIV-2, followed by differentiation of HIV-2 by a third CE-marked antibody-only test."
- "Clinicians should consider revisiting a previous diagnosis of HIV-1 by repeating HIV-2 serology and molecular tests in individuals with an undetectable HIV-1 viral load in the absence of ART, but

a falling CD4 count. This is in order to detect the possibility of missed HIV-1 and HIV-2 dual infection."

- "In those with diagnosed HIV-2 with an undetectable viral load in the absence of ART, clinicians should consider repeating HIV-1 diagnostic tests, if their CD4 count falls. This is to investigate the possibility of HIV-1 superinfection."

For the diagnosis of acute primary HIV-2

- "Investigation for acute or very recent HIV-2 infection should start as for diagnosis of chronic HIV-2 infection. A negative HIV-2 screening result on a blood sample taken within 3 months of the likely exposure should be further investigated at 6 weeks and 3 months, with parallel testing for HIV-2 viral RNA and, if necessary, HIV-2 proviral DNA."

For the investigation of indeterminate HIV-1 or HIV-2:

- "We recommend that any HIV-1 or HIV-2 serology that does not fit into a clear pattern of a confirmed laboratory diagnosis is fully investigated for the presence or absence of HIV-2 infection, and that this should be established by PCR for HIV-2 proviral DNA."

For measuring HIV-2 viral load:

- "If the pre-treatment viral load was detectable, the viral load should be measured at 1, 3 and 6 months after starting or changing ART and then 3–6 monthly.
- "If the pre-treatment viral load was undetectable, the viral load should be measured at 1 month and then 6 monthly."
- The HIV-2 viral load should be repeated in those on ART when it has been maximally suppressed and then becomes detectable."
- Testing for drug resistance should be performed in those on ART when the HIV-2 viral load has been maximally suppressed and then becomes repeatedly detectable."

For resistance testing:

- "Resistance testing should be performed at diagnosis, prior to treatment initiation and at virological failure, if the HIV-2 viral load meets the threshold of ≥500 copies/mL" (BHIVA, 2021).

European AIDS Clinical Society (EACS)

The EACS recommends a genotypic resistance test to be ideally done at the time of HIV diagnosis; testing "should not delay ART initiation (it may be re-adjusted after genotypic test results). Resistance testing is also recommended to be performed in the setting of virological failure, "preferably on failing therapy (usually routinely available for HIV-VL levels >200-500 copies/mL and in specialized laboratories for lower levels of viremia) and obtain historical resistance testing for archived mutations." For pregnant women, the EACS recommends performing resistance testing on women whose HIV-VL is not undetectable at third trimester, and "consider changing to or adding INSTI (RAL or DTG) if not on this class to obtain rapid HIV-VL decline." When considering PEP, the EACS recommends resistance testing if the HIV-VL is detectable in an HIV-positive source person on ART. They also recommend baseline resistance testing when considering

a combination regiment for ART-naïve children and adolescents living with HIV. Resistance testing should also be used to help guide the choice of treatment.

Additional genotypic recommendations include if the patient was not previously tested or if the patient is at risk of a super-infection. Genotypic resistance testing is also required prior to beginning treatment with doravirine. When switching strategies for "virologically suppressed persons," Proviral DNA genotyping may be useful in persons with multiple virological failures, unavailable resistance history or low-level viremia at the time of switch. Results ought to be taken cautiously as proviral DNA genotype may not detect previous resistance mutations and can also detect clinically irrelevant mutations. Therefore, routine proviral DNA genotyping is currently not recommended." The EACS recommends a genotypic test over a phenotypic test as genotype tests are more available and more sensitive (EACS, 2022).

The American Academy of Pediatrics (AAP)

The AAP recommends:

- "Routine HIV screening is recommended for all youth 15 years or older, at least once, in health care settings."
- "After initial screening, youth at increased risk, including sexually active youth, should be rescreened at least annually, potentially as frequently as every 3 to 6 months if at high risk (male youth reporting male sexual contact, active injection drug users, transgender youth; having sexual partners who are HIV-infected, of both genders, or injection drug users; exchanging sex for drugs or money; or those who have had a diagnosis of or request testing for other STIs)."
- "Youth who request HIV screening at any time should be tested, even in the absence of reported risk factors" (Hsu & Rakhmanina, 2022)

The Bright Futures/AAP Periodicity Schedule describes the screenings, assessments, physical examinations, procedures, and timing of anticipatory guidance recommended for each age-related visit. These guidelines provide the following recommendation for HIV screening:

- STI/HIV screening annually starting at 11 years old, with at least one HIV screening between 15 and 18 (AAP, 2022).

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends screening adolescents under 15 who are at increased risk, adolescents and adults aged 15 to 65 years, and younger adolescents and older adults who are at increased risk should.

The USPSFT also recommends screening all pregnant women for HIV, including those in labor who are untested and whose HIV status is unknown (USPSTF, 2019). The CDC recognizes and supports these guidelines (CDC, 2020).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

All three of the primary RT-PCR tests for HIV-1 have been approved by the FDA.

Human Immunodeficiency Virus (HIV), continued

In May 2007, the FDA approved the Abbott RealTime HIV-1 Amplification Reagent Kit. From the FDA website: "The Abbott RealTime HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) on the automated m2000 System in human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL". (FDA, 2007a)

On May 11, 2007, the FDA approved the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test. From the FDA website: "The COBAS AmpliPrep/COBAS TaqMan HIV-1 is an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus (HIV-1) nucleic acid in human plasma, using the COBAS AmpliPrep Instrument for automated sample preparation and the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer for automated amplification and detection. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 infected patients"(FDA, 2007b).

In 2016, the FDA approved the Aptima® HIV-1 Quant Assay. From the FDA website: "The Aptima HIV-1 Quant assay is an in vitro nucleic acid amplification test (NAAT) for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals on the fully automated Panther® system. The Aptima HIV-1 Quant assay quantitates HIV-1 RNA groups M, N, and O over the range of 30 to 10,000,000 copies/mL" (FDA, 2016). On November 20, 2020, this assay was given an FDA approval for dual use for diagnosis and viral load monitoring for HIV-1 (BusinessWire, 2020; FDA, 2020).

The following screening antibody tests are FDA-approved to differentiate HIV-1 from HIV-2.

On August 26, 2019, the FDA approved the Geenius HIV-1/2 Supplemental Assay. From the FDA Website: "The Geenius™ HIV 1/2 Supplemental Assay is a single-use immunochromatographic assay for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in serum or plasma samples (EDTA, lithium heparin, sodium citrate, and CPD) from blood donors. The Geenius™ HIV 1/2 Supplemental Assay is intended for use as an additional, more specific test for human serum and plasma samples with repeatedly reactive results by an FDA licensed blood donor screening test for antibodies to HIV-1/HIV-2. The results of the Geenius™ HIV 1/2 Supplemental Assay are read and interpreted only with the Geenius™ Reader with dedicated software." 200 known HIV-2 positive samples were classified by Geenius, with 77 interpreted as only HIV-2 positive, 108 with HIV-2 with HIV-1 cross reactivity, 12 as undifferentiated, and 3 as HIV-2 indeterminate (FDA, 2019).

On July 23, 2015, the FDA approved the BioPlex 2200 HIV Ag-Ab Assay. From the FDA Website: "The BioPlex 2200 HIV Ag-Ab assay is a multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation of the individual analytes HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies in human serum or plasma (fresh or frozen K2 EDTA, K3 EDTA, lithium heparin, sodium heparin; fresh citrate). This assay is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute (primary) HIV-1 infection. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects as young as two years of age, and

pregnant women." The test was found to differentiate all 1363 HIV-1 samples correctly and 188 of 200 HIV-2 samples correctly (with 12 "undifferentiated") (FDA, 2015).

Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86689	Antibody; HTLV or HIV antibody, confirmatory test (eg, Western Blot)
86701	Antibody; HIV-1
86702	Antibody; HIV-2
86703	Antibody; HIV-1 and HIV-2, single result
87389	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies, single result
87390	Infectious agent antigen detection by immunoassay technique (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]), qualitative or semiquantitative; HIV-1
87391	Infectious agent antigen detection by immunoassay technique (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]), qualitative or semiquantitative; HIV-2
87534	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, direct probe technique
87535	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, amplified probe technique, includes reverse transcription when performed
87536	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, quantification, includes reverse transcription when performed
87537	Infectious agent detection by nucleic acid (DNA or RNA); HIV-2, direct probe technique
87538	Infectious agent detection by nucleic acid (DNA or RNA); HIV-2, amplified probe technique, includes reverse transcription when performed
87539	Infectious agent detection by nucleic acid (DNA or RNA); HIV-2, quantification, includes reverse transcription when performed
87806	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies
87900	Infectious agent drug susceptibility phenotype prediction using regularly updated genotypic bioinformatics

87901	Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, reverse transcriptase and protease regions
87903	Infectious agent phenotype analysis by nucleic acid (DNA or RNA) with drug resistance tissue culture analysis, HIV 1; first through 10 drugs tested
87904	Infectious agent phenotype analysis by nucleic acid (DNA or RNA) with drug resistance tissue culture analysis, HIV 1; each additional drug tested (List separately in addition to code for primary procedure)
87906	Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, other region (eg, integrase, fusion)
0219U	Infectious agent (human immunodeficiency virus), targeted viral next-generation sequence analysis (ie, protease [PR], reverse transcriptase [RT], integrase [INT]), algorithm reported as prediction of antiviral drug susceptibility Proprietary test: Sentosa® SQ HIV-1 Genotyping Assay Lab/Manufacturer: Vela Diagnostics USA, Inc
G0432	Infectious agent antibody detection by enzyme immunoassay (EIA) technique, HIV-1 and/or HIV-2, screening
G0433	Infectious agent antibody detection by enzyme-linked immunosorbent assay (ELISA) technique, HIV-1 and/or HIV-2, screening
G0435	Infectious agent antibody detection by rapid antibody test, HIV-1 and/or HIV-2, screening
G0475	HIV antigen/antibody, combination assay, screening
S3645	HIV-1 antibody testing of oral mucosal transudate

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes
9/27/23	Changed title of policy from "Plasma HIV-1 and HIV-2 RNA Quantification for HIV Infection" to "Human Immunodeficiency Virus (HIV)"; also, incorporated criteria for preventive screenings and HIV genotyping and phenotyping into policy.

Prenatal Screening (Nongenetic)

Policy #: AHS – G2035

Prior Policy Name & Number (as applicable):

Implementation Date: 9/15/21

Date of Last Revision: 4/28/22, 5/11/22, 8/19/22, 10/27/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Prenatal screening encompasses any testing done to determine the health status of the pregnant individual and/or fetus. Biochemical prenatal screening encompasses screening for infectious diseases and conditions that may complicate the pregnancy. Screening refers to testing of asymptomatic or healthy individuals to search for a condition that may affect the pregnancy or individual, whereas diagnostic testing is used to either confirm or refute true abnormalities in an individual (Grant & Mohide, 1982; Lockwood & Magriples, 2023).

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

II. Indications and/or Limitations of Coverage

1. The following routine prenatal screening **MEETS COVERAGE CRITERIA** for all pregnant individuals:

   a. Screening for HIV infection

   b. Screening for Chlamydia trachomatis infection

c. Screening for Neisseria gonorrhea infection

d. Screening for hepatitis B

e. Screening for syphilis

f. Screening for hepatitis C

g. Screening for type 2 diabetes at the first prenatal visit

h. Screening for gestational diabetes during gestational weeks 24 – 28 and at the first prenatal visit if risk factors are present

i. Determination of blood type, Rh(D) status, and antibody status during the first prenatal visit, and repeated Rh (D) antibody testing for all unsensitized Rh (D)-negative individuals at 24 to 28 weeks' gestation, unless the biological father is known to be Rh (D)-negative

j. Screening for anemia with a CBC or hemoglobin and hematocrit with mean corpuscular volume

k. Screening for Group B strep once, recommended during gestational weeks 36 to 37 by American College of Obstetricians and Gynecologists (ACOG)

l. Urinalysis and urine culture

m. Rubella antibody testing

n. Testing for varicella immunity

o. Screening for tuberculosis in pregnant individuals deemed to be at high risk for TB (i.e. women with close contact with individuals with active pulmonary / respiratory tuberculosis or highly contagious active tuberculosis and women who are immunocompromised)

2. For pregnant individuals, third trimester re-screening of Chlamydia trachomatis, Neisseria gonorrhea, syphilis, and/or HIV infections **MEETS COVERAGE CRITERIA** when ANY one of the following high-risk criteria are met:

   a. For individuals under 25 years of age.

   b. For individuals with new or multiple sexual partners.

   c. For individuals with a history of sexually transmitted infections (bacterial vaginosis, chancroid, chlamydia, gonorrhea, genital herpes, hepatitis B, hepatitis C, HIV/AIDS, human papillomavirus, lymphogranuloma venereum, syphilis, trichomoniasis)

d. For individuals with past or current injection drug use

3. For pregnant individuals, fetal fibronectin (FFN) assays **MEET COVERAGE CRITERIA** for pregnant individuals when ALL the following criteria are met:

   a. Singleton or twin gestations,

   b. Intact membranes,

   c. Cervical dilation < 3 cm,

   d. The individual is experiencing symptoms suggestive of preterm labor between 24 and less than 35 weeks' gestation.

4. For individuals with a normal pregnancy without complications, human chorionic gonadotropin (hCG) hormone testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5. For all other situations not described above, FFN assays **DO NOT MEET COVERAGE CRITERIA.**

6. As a technique of risk assessment for preterm labor or delivery, serial monitoring of salivary estriol levels **DOES NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Prenatal screening is a part of overall prenatal care to promote optimal care of both mother and baby and allows for assessment and monitoring of the fetus for the presence of congenital defects or disease. Various professional medical organizations provide guidelines for prenatal screening. "Screening is an offer on the initiative of the health system or society, rather than a medical intervention in answer to a patient's complaint or health problem. Screening aims at obtaining population health gains through early detection that enables prevention or treatment (de Jong et al., 2015)."

Routine prenatal screening may include several laboratory tests, such as hematocrit or hemoglobin testing to check for anemia and possible thalassemia, pending further diagnostic testing. Blood typing and antibody screening can be performed to prevent possible alloimmunization or hemolytic disease and glucose testing can screen for possible gestational diabetes mellitus. Screening for asymptomatic bacteriuria and proteinuria is recommended as well as screening for infectious disorders, such as HIV, syphilis, chlamydia, and gonorrhea (Lockwood & Magriples, 2023).

Red blood cell antigen discrepancy between a mother and fetus may also occur during pregnancy. This is known as hemolytic disease of the fetus and newborn (HDFN), and causes maternal antibodies to destroy the red blood cells of the neonate or fetus (Calhoun, 2023). Alloimmunization is the immune response which occurs in the mother due to foreign antigens after exposure to genetically foreign cells, occurring almost exclusively in mothers with type O blood. However, while ABO blood type incompatibility is identified in almost 15% of pregnancies, HDFN is only identified in approximately 4% of pregnancies (Calhoun, 2020). Another important inherited antigen sometimes found on the surface of red blood cells is known as the Rhesus (Rh)D antigen. During pregnancy and delivery, individuals who are RhD negative may be exposed to RhD positive fetal cells, which can lead to the development of anti-RhD antibodies. This exposure typically happens during delivery and affects subsequent pregnancies; infants with RhD incompatibility tend to experience a more severe form of HDFN than those with ABO incompatibility (Calhoun, 2020). The clinical presentation of HDFN may be mild (such as hyperbilirubinemia with mild to moderate anemia) to severe and life-threatening anemia (such as hydrops fetalis) (Calhoun, 2020). Less severely affected infants may develop hyperbilirubinemia within the first day of life; infants with RhD HDFN may also present with symptomatic anemia requiring a blood transfusion. In more severe cases, infants with severe life-threatening anemia, such as hydrops fetalis, may exhibit shock at delivery requiring an emergent blood transfusion (Calhoun, 2020).

The administration of anti-D immune globulin has been able to dramatically reduce, but not eliminate, the number of RhD alloimmunization cases. "Anti-D immune globulin is manufactured from pooled plasma selected for high titers of IgG antibodies to D-positive erythrocytes (Moise Jr., 2022)." Before the development of this anti-D immune globulin, it has been reported that 16% of RhD-negative individuals with two deliveries of RhD positive ABO compatible infants became alloimmunized. However, this rate falls to 1-2% with routine postpartum administration of a single dose of anti-D immune globulin. An additional administration in the third trimester of pregnancy, further reduces the incidences of alloimmunization to 0.1-0.3% (Moise Jr., 2022).

Human chorionic gonadotropin (hCG) is a biomarker in the glycoprotein hormone family. Other hormones in this family include luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid stimulating hormone. hCG in pregnancy serves as an important biomarker for the detection of pregnancy-related disorders and hCG is also measured in some prenatal tests for Down syndrome. Low levels of hCG are associated with pregnancy loss and preeclampsia, while high levels can be associated with Down syndrome pregnancies (Richard Alan Harvey, 2023) A qualitative hCG test may be used to screen for pregnancy and gives a simple positive or negative result. A quantitative hCG measurement is used to assess pregnancy viability and screen for disorders. Quantitative hCG tests measures the exact amount of hCG in blood; for example, during 10-12 weeks of gestation, hCG levels are expected to approximately double every 24-48 hours, such that abnormal measurement results for hCG may indicate issues with the pregnancy (AACC, 2023).

Clinical Utility and Validity

Education and counseling are a key factor in prenatal screening and diagnostic tests. Yesilcinar and Guvenc (2021) found that a proactive intervention approach decreased anxiety and decisional conflict in the pregnant individual and increased attitudes towards the tests, having a positive effect on the pregnant individual's knowledge level and decision satisfaction. This allowed the individual to make more informed decisions, such as opting to have screening and diagnostic testing performed. (Yesilcinar & Guvenc, 2021)

Implementation of prenatal screening tests can positively affect pregnancies and pregnancy outcomes. The Centers for Disease Control and Prevention (CDC) reports that implementation of the 1996 guidelines concerning Group B Streptococcus (GBS) had a profound effect. Prior to screening and widespread use of intrapartum antibiotics, invasive neonatal GBS occurred in 2 - 3 cases per 1,000 live births; however, after prenatal screening implementation, the rate declined to 0.5 cases per 1,000 live births in 1999 (Schrag, Gorwitz, Fultz-Butts, & Schuchat, 2002). The CDC also reports from a multi-year study that screening for syphilis in all pregnant individuals at the first prenatal visit (and then rescreening in third trimester for individuals at risk) is very important in preventing congenital syphilis, which can cause spontaneous abortion, stillbirth, and early infant death. They show that 88.2% of cases of congenital syphilis was avoided when proper screening was applied; moreover, 30.9% of the cases of congenital syphilis that did occur happened when the mother did not receive proper prenatal care (≥45 days before delivery) (Slutsker, et al., 2018).

IV. Guidelines and Recommendations

American College of Obstetricians and Gynecologists (ACOG)

ACOG has several practice guidelines related to prenatal care as well as both pre-conception and prenatal testing. ACOG recommendations and guidelines include the following:

- **Vitamin D Screening:** Concerning vitamin D screening, "there is insufficient evidence to support a recommendation for screening all pregnant women for vitamin D deficiency. For pregnant women thought to be at increased risk of vitamin D deficiency, maternal serum 25-hydroxyvitamin D levels can be considered and should be interpreted in the context of the individual clinical circumstance" (ACOG, 2011). This was reaffirmed in 2021.
- **Lead Screening:** Concerning lead screening, ACOG recommends "evaluating risk factors for exposure as part of a comprehensive health risk assessment and perform blood lead testing if a single risk factor is identified. Assessment of lead exposure should take place at the earliest contact with the pregnant patient" (ACOG, 2012). This position was reaffirmed in 2023.
- **Subclinical Hypothyroidism:** ACOG Committee Opinion on subclinical hypothyroidism in pregnancy does not recommend routine screening for subclinical hypothyroidism. It states that "thyroid testing in pregnancy should be performed on symptomatic women and those with a personal history of thyroid disease or other medical conditions associated with thyroid disease (e.g., diabetes mellitus) (ACOG, 2015a)."
- **Depression and Anxiety:** "All obstetrician-gynecologists and other obstetric care providers screen patients at least once during the perinatal period for depression and anxiety symptoms using a standardized, validated tool. [They should] complete a full assessment of mood and emotional well-being (including screening for postpartum depression and anxiety with a validated instrument) during the comprehensive postpartum visit for each patient (ACOG, 2018a). If a patient is screened for depression and anxiety during pregnancy, additional screening should then occur during the comprehensive postpartum visit" (ACOG, 2018b).
- ***Listeria monocytogenes:*** Concerning testing for *Listeria monocytogenes (ACOG, 2014)*, "No testing, including blood and stool cultures, or treatment is indicated for an asymptomatic pregnant woman

who reports consumption of a product that was recalled or implicated during an outbreak of listeria contamination. An asymptomatic patient should be instructed to return if she develops symptoms of listeriosis within 2 months of eating the recalled or implicated product" (ACOG, 2014). If an exposed pregnant woman shows signs and symptoms consistent with infection, then blood culture testing is the standard of care. Stool culture testing is not recommended since it has not been validated as a screening tool. (ACOG, 2014). This position was reaffirmed in 2023.

- **HIV:** Concerning HIV, ACOG recommends that all women should be tested for HIV with the right to refuse testing. "Human immunodeficiency virus testing using the opt-out approach, which is currently permitted in every jurisdiction in the United States, should be a routine component of care for women during prepregnancy and as early in pregnancy as possible. Repeat HIV testing in the third trimester, preferably before 36 weeks of gestation, is recommended for pregnant women with initial negative HIV antibody tests who are known to be at high risk of acquiring HIV infection; who are receiving care in facilities that have an HIV incidence in pregnant women of at least 1 per 1,000 per year; who are incarcerated; who reside in jurisdictions with elevated HIV incidence; or who have signs and symptoms consistent with acute HIV infection (e.g., fever, lymphadenopathy, skin rash, myalgias, arthralgias, headache, oral ulcers, leukopenia, thrombocytopenia, or transaminase elevation). Rapid screening during labor and delivery or during the immediate postpartum period using the opt-out approach should be done for women who were not tested earlier in pregnancy or whose HIV status is otherwise unknown. Results should be available 24 hours a day and within 1 hour (ACOG, 2018a)."
    - For pregnant women who test positive for HIV, "Additional laboratory work, including $CD4^+$ count; HIV viral load; testing for antiretroviral resistance; hepatitis C virus antibody; hepatitis B surface antigen and viral load; and hepatitis A using antibody testing for immunoglobulin G for women who have hepatitis B virus infection and who have not already received the hepatitis A virus vaccine series; complete blood count with platelet count; and baseline chemistries with comprehensive metabolic testing, will be useful before prescribing antiretroviral therapy (Pollock et al., 2019)."

    - **Prevention of Rh D Alloimmunization:** Concerning the prevention of Rh D alloimmunization, ACOG has published the guidelines supporting the administration of anti-D immune globulin to individuals in various scenarios. However, these guidelines do not mention the use of cell-free fetal DNA for fetal RHD testing to determine if anti-D immune globulin is needed (ACOG, 2017).

- **Group B Streptococcal (GBS) Disease:** "All pregnant women should undergo antepartum screening for GBS at 36 0/7–37 6/7 weeks of gestation, unless intrapartum antibiotic prophylaxis for GBS is indicated because of GBS bacteriuria during the pregnancy or because of a history of a previous GBS-infected newborn. This new recommended timing for screening provides a 5-week window for valid culture results that includes births that occur up to a gestational age of at least 41 0/7 weeks" (ACOG, 2020)

- **Lab Tests:** ACOG lists the following lab tests to be performed early in pregnancy: complete blood count (CBC), blood type and Rh factor, urinalysis, urine culture, rubella, hepatitis B, hepatitis C, HIV, sexually transmitted infection (STI) testing, and tuberculosis. (ACOG, 2021).

- ACOG lists the following lab tests to be performed later in pregnancy: repeat CBC, Rh antibody test, glucose screening test, and Group B streptococci (GBS) (ACOG, 2021).

United States Preventive Services Task Force (USPSTF)

The United States Preventive Services Task Force (USPSTF) recommends the following testing for pregnant women:

- Screening for gestational diabetes in asymptomatic pregnant individuals at ≥ 24 weeks of gestation (Grade B) (Force, 2021).
- Screening for hepatitis B virus (HBV) infection at the first prenatal visit (Grade A) (Force, 2019c).
- Screening for asymptomatic bacteriuria with urine culture is recommended in pregnant persons (Grade B) (Force, 2019a).
- Screening for HIV is recommended in all pregnant persons, including those in labor or whose HIV status is unknown at delivery (Grade A) (Force, 2019d).
- Rh (D) blood typing and antibody testing for all pregnant individuals during their first visit for pregnancy-related care (Grade A) (USPSTF, 2005).
- Repeated Rh (D) antibody testing for all unsensitized Rh (D)-negative individuals at 24-28 weeks' gestation, unless the biological father is known to be Rh (D)-negative (Grade B) (USPSTF, 2005).

Additional recommendations from the USPSTF that may be relevant during pregnancy include:

- Screening for chlamydia in sexually active women aged 24 years or younger and in older women who are at increased risk for infection (Grade B) (LeFevre & USPSTF, 2014)

- Screening for gonorrhea in sexually active women aged 24 years or younger and in older women who are at increased risk for infection (Grade B) (LeFevre & USPSTF, 2014)

- Screening for depression in general population, including pregnant and post-partum women (Grade B) (Siu & USPSTF, 2016)

- Screening for hepatitis C virus (HCV) infection is recommended in all adults aged 18 to 79 years (Grade B) (Chou et al., 2020; V. A. Moyer & USPSTF, 2013a)

- Concerning screening adults for drug use, Krist et al. (2020) state that "The USPSTF recommends screening by asking questions about unhealthy drug use in adults age 18 years or older. Screening should be implemented when services for accurate diagnosis, effective treatment, and appropriate care can be offered or referred. (Screening refers to asking questions about unhealthy drug use, not testing biological specimens.)" The USPSTF also states that "This new evidence supports the current recommendation that primary care clinicians offer screening to adults 18 years or older, including those who are pregnant or postpartum, when services for accurate diagnosis, effective treatment, and appropriate care can be offered or referred." (Krist et al., 2020)

- However, the USPSTF recommends against the following tests during pregnancy:
    - Screening for bacterial vaginosis in pregnant women who are not at risk for preterm delivery (grade D); further, current evidence is insufficient for screening pregnant persons who are at increased risk for preterm delivery (Force, 2020):
    - Serological screening for herpes simplex virus (HSV) in asymptomatic pregnant women (USPSTF, 2016)
    - Screening for elevated blood lead levels in asymptomatic pregnant women has been given an I recommendation as current evidence is insufficient to determine if this testing is beneficial or not (Force, 2019b)
    - "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in pregnant women to prevent adverse maternal health and birth outcomes (Siu, 2015)."

American Diabetes Association (ADA)

The American Diabetes Association in the 2018 *Standards of Medicare Care in Diabetes* make the following recommendations (ADA, 2018, 2020):

- "Starting at puberty and continuing in all [individuals] with diabetes and reproductive potential, preconception counseling should be incorporated into routine diabetes care. [Grade] **A**
- Preconception counseling should address the importance of achieving glucose levels as close to normal as is safely possible, ideally A1C <6.5% (48 mmol/mol), to reduce the risk of congenital anomalies, preeclampsia, macrosomia, preterm birth, and other complications. [Grade] **B**
- Women with preexisting diabetes who are planning a pregnancy should ideally be managed beginning in preconception in a multidisciplinary clinic including an endocrinologist, maternal-fetal medicine specialist, registered dietitian nutritionist, and diabetes care and education specialist, when available. [Grade] **B**
- Women with preexisting type 1 or type 2 diabetes who are planning pregnancy or who have become pregnant should be counseled on the risk of development and/or progression of diabetic retinopathy. Dilated eye examinations should occur ideally before pregnancy or in the first trimester, and then patients should be monitored every trimester and for 1 year postpartum as indicated by the degree of retinopathy and as recommended by the eye care provider. [Grade] **B**

- "Test for undiagnosed prediabetes at the first prenatal visit in those with risk factors, using standard diagnostic criteria. [Grade] **B**
- Test for gestational diabetes mellitus at 24–28 weeks of gestation in pregnant women not previously found to have diabetes. [Grade] **A**
    - Screen women with a recent history of gestational diabetes mellitus at 4–12 weeks postpartum, using the 75-g oral glucose tolerance test and clinically appropriate nonpregnancy diagnostic criteria [Grade] **B**

- Women with a history of gestational diabetes mellitus should have lifelong screening for the development of type 2 diabetes or prediabetes every 1–3 years. [Grade] **B**
- Women with a history of gestational diabetes mellitus found to have prediabetes should receive intensive lifestyle interventions and/or metformin to prevent diabetes. [Grade] **A**
- Women with a history of gestational diabetes mellitus should seek preconception screening for diabetes and preconception care to identify and treat hyperglycemia and prevent congenital malformations. [Grade] **E**"
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Centers for Disease Control and Prevention (CDC)
The Centers for Disease Control and Prevention (CDC) recommends:

- All pregnant women get testing for HIV, hepatitis B virus (HBV) and syphilis during each pregnancy (CDC, 2019b). Additional CDC (2019b) recommendations can be found in the table below:

	First Prenatal Visit	Third Trimester	At Delivery
Syphilis	All pregnant women	Certain groups of pregnant women[v] at 28-32 weeks	Certain groups of pregnant women,[v] at delivery
HIV	All pregnant women[i]	Certain groups of pregnant women[vi] before 36 weeks	Pregnant women not screened during pregnancy
HBV	All pregnant women[ii]	Rescreen women at high risk for acquiring HIV infection	Pregnant women not screened during pregnancy,[vii] who are at high risk,[viii] or with signs or symptoms of hepatitis
Chlamydia	All pregnant women <25 years of age and older pregnant women at increased risk[iii]	Pregnant women <25 years of age or continued high risk[iii]	N/A
Gonorrhea	All pregnant women <25 years of age and older pregnant women at increased risk[iii]	Pregnant women at continued high risk[iii]	N/A
Hepatitis C (HCV)	All[8] pregnant women during each pregnancy	N/A	N/A

"Endnotes:

1. To promote informed and timely therapeutic decisions, health care providers should test women for HIV as early as possible during each pregnancy.[1]

2. All pregnant women should be tested for hepatitis B surface antigen (HBsAg) during an early prenatal visit (e.g., first trimester) in each pregnancy, even if they have been vaccinated or tested previously.[2]
3. "Increased risk" means new or multiple sex partners, sex partner with concurrent partners, sex partners who have a sexually transmitted disease (STD).[3,4]
4. "At increased risk" means living in a high-morbidity area, having a previous or coexisting STI, new or multiple sex partners, inconsistent condom use among persons not in mutually monogamous relationships, exchanging sex for money or drugs.[3]
5. "Certain groups" includes women who are at high risk for syphilis during pregnancy, who live in areas with high numbers or syphilis cases, and/or who were not previously tested or had a positive test in the first trimester.[3]
6. Women admitted for delivery at a health care facility without documentation of HBsAg test results should have blood drawn and tested as soon as possible after admission.[2]
7. Having had more than one sex partner during the previous 6 months, an HBsAg-positive sex partner, evaluation or treatment for a STD, or injection-drug use IDU[2]."
8. All pregnant women except in a setting where the prevalence of HCV infection is (HCV RNA-positivity) < 0.1%." (CDC, 2021a)

- A second test during the third trimester, preferably at <36 weeks' gestation, should be considered and is recommended for [individuals] who are at high risk for acquiring HIV infection, [individuals] who receive health care in jurisdictions with high rates of HIV, and [individuals] examined in clinical settings in which HIV incidence is ≥1 per 1,000 [individuals] screened per year" (CDC, 2021f).

- "Regardless of whether they have been previously tested or vaccinated, all pregnant [individuals] should be tested for HBsAg at the first prenatal visit and again at delivery if at high risk for HBV infection (see STI Detection Among Special Populations). Pregnant [individuals] at risk for HBV infection and without documentation of a complete hepatitis B vaccine series should receive hepatitis B vaccination" (CDC, 2021d).

- "[individuals] aged <25 years and those at increased risk for chlamydia (i.e., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI) should be screened at the first prenatal visit and rescreened during the third trimester to prevent maternal postnatal complications and chlamydial infection in the infant" (CDC, 2021b).

- "Annual screening for *N. gonorrhoeae* infection is recommended for all sexually active [individuals] aged <25 years and for older [individuals] at increased risk for infection (e.g., those aged ≥25 years who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI . . . [All individuals] who have been treated for gonorrhea should be retested 3 months after treatment regardless of whether they believe their sex partners were treated" (CDC, 2021c).

- "CDC recommends hepatitis C screening . . . all [individuals] during each pregnancy, except in settings where the prevalence of HCV infection is <0.1%" (CDC, 2021e).

Zika virus testing for asymptomatic individuals is not currently recommended. For symptomatic pregnant individuals:

- "For symptomatic pregnant [individuals] who had recent travel to areas with active dengue transmission and a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset.

- The following diagnostic testing should be performed at the same time:
  - Dengue and Zika virus NAAT testing on a serum specimen, and Zika virus NAAT on a urine specimen, and
  - IgM testing for dengue only.
- Zika virus IgM testing is NOT recommended for symptomatic pregnant [individuals].
  - Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.
  - There is notable cross-reactivity between dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive.
- If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results. If the dengue NAAT is positive, this provides adequate evidence of a dengue infection, and no further testing is indicated.
- If the IgM antibody test for dengue is positive, this is adequate evidence of a dengue infection and no further testing is indicated" (CDC, 2019).

- "Evidence does not support routine HSV-2 serologic testing among asymptomatic pregnant [individuals]" (CDC, 2021a).
- "Evidence does not support routine screening for BV in asymptomatic pregnant [individuals] at high or low risk for preterm delivery" (CDC, 2021a).

To help circumvent prenatal transmission, the CDC also "recommends that all pregnant [individuals] get tested for HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis during each pregnancy" as "screening is necessary to access medical services for HCV and treatment to prevent transmission of HIV, HBV, and syphilis to the infant" (CDC, 2020).

American College of Medical Genetics and Genomics (ACMG)

In 2014, the ACMG released guidelines concerning the diagnosis and management of phenylalanine hydroxylase (PAH) deficiency. They recommend PAH testing be part of newborn screening and that quantitative blood amino acids testing should be performed for diagnostic testing following a positive newborn screen of PAH deficiency. "Additional testing is needed to define the cause of elevated PHE and should include analysis of pterin metabolism; PAH genotypic is indicated for improved therapy planning (Vockley et al., 2014)."

World Health Organization (WHO)

In 2016, the WHO released their publication titled, *WHO recommendations on antenatal care for a positive pregnancy experience*, which had the following recommendations (WHO, 2016):

- Anemia (Context-specific recommendation)—"Full blood count testing is the recommended method for diagnosing anaemia in pregnancy."
- Asymptomatic bacteriuria (Context-specific recommendation)—"Midstream urine culture is the recommended method for diagnosing asymptomatic bacteriuria (ASB) in pregnancy. In settings

where urine culture is not available, on-site midstream urine Gram-staining is recommended over the use of dipstick tests as the method for diagnosing ASB in pregnancy."

- Gestational diabetes mellitus (Recommended)—"Hyperglycaemia first detected at any time during pregnancy should be classified as either gestational diabetes mellitus (GDM) or diabetes mellitus in pregnancy, according to WHO criteria."
- HIV and syphilis (Recommended)—"In high-prevalence settings, provider-initiated testing and counselling (PITC) for HIV should be considered a routine component of the package of care for pregnant women in all antenatal care settings. In low-prevalence settings, PITC can be considered for pregnant women in antenatal care settings as a key component of the effort to eliminate mother-to-child transmission of HIV, and to integrate HIV testing with syphilis, viral or other key tests, as relevant to the setting, and to strengthen the underlying maternal and child health systems."
- Tuberculosis (Context-specific recommendation)—"In settings where the tuberculosis (TB) prevalence in the general population is 100/100 000 population or higher, systematic screening for active TB should be considered for pregnant women as part of antenatal care (WHO, 2016)."

Department of Veterans Affairs/Department of Defense (VA/DoD)

In the 3rd edition of the VA/DoD *Clinical Practice Guideline for the Management of Pregnancy (VA & DOD, 2018)*, they list the following lab tests as routine for all pregnancies in the first prenatal visit: HIV, CBC, ABO Rh blood typing, Antibody screen, anemia/hemoglobinopathies screen, rapid plasma 38eagin, gonorrhea, chlamydia, hepatitis B surface antigen test, rubella IgG, Urinalysis and culture, and varicella IgG (if status is unknown). They also list the following among their recommendations (VA & DOD, 2018):

- "We recommend screening for use of tobacco, alcohol, illicit drugs, and unauthorized use of prescription medication because their use is common and can result in adverse outcomes. For women who screen positive, we recommend additional evaluation and treatment." [Strong]
- "We recommend screening for depression using a standardized tool such as the Edinburgh Postnatal Depression Scale or the 9- item Patient Health Questionnaire periodically during pregnancy and postpartum." [Strong]
- "We suggest making prenatal diagnostic testing for aneuploidy available to all pregnant women." [Weak]
- "We recommend offering prenatal screening for aneuploidy and the most common clinically significant genetic disorders to all pregnant women. When aneuploidy screening is desired, cellfree fetal DNA screening should be considered; however, screening test selection should be individualized and take into account the patient's age, baseline aneuploidy risk, and test performance for a given condition." [Strong]
- "We suggest the two-step process (one-hour oral glucose challenge test followed by three-hour oral glucose tolerance test) to screen for gestational diabetes mellitus at 24-28 weeks gestation for all pregnant women." [Weak]

- "We suggest that pregnant women with an unexplained elevation of maternal serum alpha-fetoprotein be evaluated and counseled by a qualified obstetric provider due to increased risk for adverse perinatal outcomes." [Weak]
- "We recommend **against** routine screening for preterm delivery using the fetal fibronectin test in asymptomatic women." [Strong, against]
- "We recommend considering the use of fetal fibronectin testing as a part of the evaluation strategy in women between 24 and 34 6/7 weeks gestation with signs and symptoms of preterm labor, particularly in facilities where the result might affect management of delivery." [Strong]
- "We suggest that women who have undergone bariatric surgery should be evaluated for nutritional deficiencies and need for nutritional supplementation where indicated (e.g., vitamin B12, folate, iron, calcium)." [Weak]

Health Resources & Services Administration (HRSA)

The HRSA recommends the following:

- Screening pregnant women for gestational diabetes mellitus after 24 weeks of gestation (preferably between 24 and 28 weeks of gestation).
- Women with risk factors for diabetes mellitus be screened for preexisting diabetes before 24 weeks of gestation—ideally at the first prenatal visit.
- Screening for HIV is recommended for all pregnant [individuals] upon initiation of prenatal care with retesting during pregnancy based on risk factors.
- Rapid HIV testing is recommended for pregnant [individuals] who present in active labor with an undocumented HIV status" HRSA (2022).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA has approved many tests for conditions that can be included in a prenatal screening, such as HSV, chlamydia, gonorrhea, syphilis, and diabetes. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
80055	Obstetric panel (must include CBC, HbSAg, Rubella antibody, RBC antibody screen, qualitative non-treponemal antibody syphilis test, ABO blood typing and Rh D typing)
80081	Obstetric panel (includes HIV testing)
81001	Urinalysis, by dip stick or tablet reagent for bilirubin, glucose, hemoglobin, ketones, leukocytes, nitrite, pH, protein, specific gravity, urobilinogen, any number of these constituents; automated, with microscopy
81002	Urinalysis, by dip stick or tablet reagent for bilirubin, glucose, hemoglobin, ketones, leukocytes, nitrite, pH, protein, specific gravity, urobilinogen, any number of these constituents; non-automated, without microscopy
81003	Urinalysis, automated, without microscopy
81007	Urinalysis, bacteriuria screen, except by culture or dipstick
81015	Urinalysis; microscopic only
81244	FMR1 (Fragile X mental retardation 1) gene analysis; characterization of alleles (e.g., expanded size and methylation status)
82677	Estriol
82731	Fetal fibronectin, cervicovaginal secretions, semi quantitative
82947	Glucose; quantitative, blood (except regent strip)
82950	Glucose; post glucose dose (includes glucose)
82951	Glucose; tolerance test (GTT), 3 specimens (includes glucose)
82962	Glucose, blood by glucose monitoring device(s) cleared by the FDA specifically for home use
83020	Hemoglobin fractionation and quantitation; electrophoresis (e.g., A2, S, C, and/or F)
83021	Hemoglobin fractionation and quantitation; chromatography (e.g., A2, S, C, and/or F)
83036	Hemoglobin, glycosylated (A1C)
84702	Gonadotropin, chorionic (hCG); quantitative

84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
85004	Blood count; automated differential WBC count
85007	Blood smear, microscopic examination with manual differential WBC count
85009	Blood Count
85014	Hematocrit (Hct)
85018	Hemoglobin (Hgb)
85025	Complete (CBC), automated (Hgb, Hct, RBC, WBC and platelet count) and automated differential WBC count
85027	Complete (CBC), automated (Hgb, Hct, RBC, WBC and platelet count)
85032	Blood count; manual cell count (erythrocyte, leukocyte, or platelet) each
85041	Blood count; red blood cell (RBC), automated
85048	Blood count; leukocyte (WBC), automated
86480	Tuberculosis test, cell mediated immunity antigen response measurement; gamma interferon
86580	Skin test; tuberculosis, intradermal
86592	Syphilis test, non-treponemal antibody; qualitative (e.g., VDRL, RPR, ART)
86593	Syphilis test, non-treponemal antibody; quantitative
86631	Antibody; Chlamydia
86632	Antibody; Chlamydia, IgM
86701	Antibody, HIV-1
86702	Antibody, HIV-2
86703	Antibody, HIV-1 and HIV-2, single result
86704	Hepatitis B core antibody (HBcAb); total
86706	Hepatitis B surface antibody (HBsAb)
86762	Rubella Antibody
86787	Antibody; varicella-zoster

86780	Antibody; Treponema pallidum
86803	Hepatitis C antibody
86804	Hepatitis C antibody; confirmatory test (e.g., immunoblot)
86850	Antibody screen, RBC, each serum technique
86900	Blood typing; ABO
86901	Blood typing; Rh (D)
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87081	Culture, presumptive, pathogenic organisms, screening only
87086	Culture, bacterial; quantitative colony count, urine
87088	Culture, bacterial; with isolation and presumptive identification of each isolate, urine
87110	Culture, chlamydia, any source
87270	Infectious agent antigen detection by immunofluorescent technique; Chlamydia trachomatis
87320	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis B surface antigen (HBsAg)
87340	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis B surface antigen (HBsAg)
87341	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis B surface antigen (HBsAg) neutralization
87490	Chlamydia trachomatis, direct probe technique
87491	Chlamydia trachomatis, amplified probe technique
87590	Neisseria gonorrhea, direct probe technique
87591	Neisseria gonorrhea, amplified probe technique

87592	Neisseria gonorrhea, quantification
87653	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group B, amplified probe technique
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique
87802	Infectious agent antigen detection by immunoassay with direct optical (i.e., visual) observation; Streptococcus, group B
87810	Infectious agent antigen detection by immunoassay with direct optical (i.e., visual) observation; Chlamydia trachomatis
87850	Infectious agent antigen detection by immunoassay with direct optical (i.e., visual) observation; Neisseria gonorrhoeae
G0306	Complete CBC, automated (HgB, HCT, RBC, WBC, without platelet count) and automated WBC differential count
G0307	Complete (CBC), automated (HgB, HCT, RBC, WBC; without platelet count)
G0432	Infectious agent antibody detection by enzyme immunoassay (EIA) technique, HIV-1 and/or HIV-2, screening
G0433	Infectious agent antibody detection by enzyme-linked immunosorbent assay (ELISA) technique, HIV-1 and/or HIV-2, screening
G0435	Infectious agent antigen detection by rapid antibody test of oral mucosa transudate, HIV-1 or HIV-2, screening
G0472	Hepatitis C antibody screening, for individual at high risk and other covered indication(s)
S3652	Saliva test, hormone level; to assess preterm labor risk
0167U	Gonadotropin, chorionic (hCG), immunoassay with direct optical observation, blood Proprietary test: ADEXUSDx hCG Test Lab/Manufacturer: NOWDiagnostics

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes
4/28/22	The following modifications were implemented: changed woman/women to individual/individuals throughout coverage criteria; unified all references to carrier screening vs some saying screening and some testing; reworded the following coverage criteria to eliminate ethnicity specific phrases: For pregnant individuals and those persons seeking pre-conception care, any of the following testing* (See Note 1 below) of carrier status **MEETS COVERAGE CRITERIA:** a. Carrier screening for cystic fibrosis is in accordance with Avalon policies M2017-Genetic Testing for Cystic Fibrosis b. screening for Canavan disease, Tay-Sachs disease, familial dysautonomia, Gaucher disease, Fanconi Anemia, Niemann-Pick type A, Bloom syndrome and mucolipidosis IV c. Carrier screening for Fragile X syndrome d. Carrier screening for spinal muscular atrophy e. Carrier screening for hemoglobinopathies and/or thalassemia f. Carrier screening for hereditary hearing loss mutations (GJB2, GJB6, and other hereditary hearing loss-related mutations) in parents according to the policy AHS-G2148-Genetic Testing for Hereditary Hearing Loss g. Carrier screening for other genetic disorders when there is a family history of a genetic disorder and a properly validated test is available. When there is a known familial mutation, testing should be limited to that mutation, when possible. (See General Genetic Testing policy for

5/11/22	more details on appropriate criteria for genetic testing.) h. Next generation sequencing (NGS) panel testing as long as a single appropriate AMA genetic sequencing procedure test code is submitted; removed "Carrier screening should be limited to once per lifetime per disorder for which the individual is at risk" from the following coverage criteria: Carrier screening* (See Note 1 below) of the biological father **MEETS COVERAGE CRITERIA** when the biological mother is known or found to be a carrier of a recessively inherited disorder. Carrier testing limitations: a. Repeat carrier screening for the same disorder does not meet coverage criteria; removed the following CC and replaced with "Testing pregnant individuals for thyroid dysfunction is covered in accordance with Avalon Policy AHS-G2045-Thyroid Disease Testing": Testing pregnant women for thyroid dysfunction **MEETS COVERAGE CRITERIA** if they have any of the following: a. Symptoms of thyroid disease b. Personal history of thyroid disease c. Personal history of other medical conditions associated with thyroid disease (e.g. diabetes mellitus, goiter, iodine deficiency); and removed CPT codes 81220, 81252, 81253, S3844, 81336, and 81337. Removed all criteria involving genetic testing and carrier screening (previously #3 through #5, and #8 through #11), as coverage for these tests is currently determined by AIM Specialty Health. Also, removed CPT code 81443.
8/19/22	Modified title of policy (added "nongenetic" to title), removed all genetic related criteria and codes from policy, and updated overall criteria to

	align with current clinical standards. Also, added CPT codes 86704 and 86706.
10/27/23	Added new coverage criteria #4: "For individuals with a normal pregnancy without complications, human chorionic gonadotropin (hCG) hormone testing **DOES NOT MEET COVERAGE CRITERIA.**"

G2035 Prenatal Screening

Prenatal Testing for Fetal Aneuploidy

Policy #: AHS – G2055	**Prior Policy Name & Number (as applicable):** • AHS-G2055-20161212-Detection of Down Syndrome Using Maternal Serum Markers • AHS-M2016-20170320-Non-Invasive Prenatal Screening for Aneuploidy
Implementation Date: 9/15/21	**Date of Last Revision:** 3/20/23, 5/19/23 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Aneuploidy is defined as an abnormal number of chromosomes present in the cell. Fetal aneuploidy is a condition where the fetus has one or more extra or missing chromosomes leading to either a nonviable pregnancy, offspring that may not survive after birth, or surviving newborn with congenital birth defects and functional abnormalities. The most common fetal aneuploidies associated with an additional chromosome are Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13). Prenatal screening for fetal aneuploidy is an assessment of the pregnant individual's risk of carrying a fetus with fetal aneuploidy using markers found in maternal serum (ACOG, 2016). Non-invasive prenatal screening is a method for screening for chromosomal abnormalities using a maternal blood sample where cell-free fetal DNA (cff-DNA) is extracted and screened for aneuploidies (McKanna et al., 2018).

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening
AHS-M2033	Chromosomal Microarray

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For pregnant individuals who are adequately counseled and desire information on the risk of having a child with fetal aneuploidy, the following screening tests to detect fetal aneuploidy of chromosomes 13, 18, and 21 **MEET COVERAGE CRITERIA:**

   a) First-trimester (defined as 11-14 weeks) screening incorporating maternal serum markers (hCG, PAAP-A with NT).

   b) Second-trimester (15-22 weeks) screening incorporating triple maternal serum markers (hCG, AFP, uE3 with NT) and quad maternal serum markers (hCG, AFP, uE3, DIA with NT).

   c) First (11-14 weeks) and second (15-22 weeks) trimester integrated screening incorporating maternal serum markers (PAPP-A with NT) and quad maternal serum markers (hCG, AFP, uE3, DIA with NT).

   d) First (11-14 weeks) and second (15-22 weeks) trimester sequential screening incorporating maternal serum markers (PAPP-A, hCG with NT) and quad maternal serum markers (hCG, AFP, uE3, DIA with NT).

   e) First (11-14 weeks) and second (15-22 weeks) trimester contingent screening incorporating maternal serum markers (PAPP-A, hCG with NT) if positive, quad maternal serum markers (hCG, AFP, uE3, DIA with NT).

2. To detect fetal aneuploidy, the use of the "penta" screen (hCg, AFP, uE3, DIA with NT, and hyperglycosylated hCG **DOES NOT MEET COVERAGE CRITERIA.**

3. Screening for the detection of fetal aneuploidies **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:

   a) Parallel or simultaneous testing with multiple screening methodologies for fetal aneuploidy.

b) For the screening of pregnant individuals with multiple gestation pregnancies, any testing other than nuchal translucency and/or subsequent diagnostic testing via CVS or amniocentesis due to the risk of high false positive results.

c) Repeat screening for pregnant individuals with negative screening results.

d) For screening in egg donor pregnancies.

e) For the detection of other chromosomal abnormalities, such as microdeletion syndromes, unbalanced translocations, deletions, and duplications, not addressed above.

f) For the determination of fetal sex.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4. For the diagnosis of fetal aneuploidy, the use of single cell genotyping in trophoblasts isolated from maternal serum (e.g., Luna Prenatal Test) **DOES NOT MEET COVERAGE CRITERIA.**

IV. Scientific Background

Pregnant individuals are routinely offered blood-based screening or invasive diagnostic testing for identification of the most common fetal aneuploidies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome). Approximately 90% of chromosomal abnormalities are due to an incorrect number of chromosomes, especially in the three aforementioned triploid conditions as well as monosomy X (Turner syndrome). Approximately 15-20% of clinically recognized pregnancies result in first trimester spontaneous abortions, with 50% of these spontaneous abortions due to chromosomal abnormalities (Witters et al., 2011).

Historically, non-invasive blood-based aneuploidy screening has taken the form of first- and/or second-trimester analysis of biomarkers in maternal circulation, sometimes along with ultrasound measurement of fetal nuchal translucency (NT). Although both the sensitivity (detection rate) and specificity (true positive rate) of maternal serum screening tests for aneuploidy have improved significantly over time, the false positive rate (2-5%) remains higher than desirable. The detection rate of Down syndrome in the first trimester using a combination of NT and biochemical markers is typically 79% - 90% (Dey et al., 2013). Positive maternal serum screen results are usually followed by an invasive diagnostic test, such as karyotyping of a chorionic villus sample (in first trimester) or karyotyping of an amniotic fluid sample (second trimester).

Additionally, detection rates of maternal serum screens are typically below 99%, resulting in the inability of a normal result to confer complete confidence that the fetus is unaffected with aneuploidy (Dey et al., 2013). Thus, many pregnant individuals who are in a high-risk category due to age or other factors may opt for the more definitive, diagnostic, invasive testing, which has its own risks and relatively high costs. The availability of non-invasive testing may improve both the sensitivity and specificity of aneuploidy detection while resulting in fewer invasive procedures, less risk, and less overall cost.

Screening Tests

Chromosomal anomalies are a leading cause of perinatal mortality and developmental abnormality. The goal of prenatal testing is to screen for chromosomal anomalies and to provide genetic counseling for parents. The American College of Obstetrics and Gynecology (ACOG) recommends that prenatal testing is offered to all pregnant individuals (ACOG, 2016, 2020). Invasive testing, including chorionic villi sampling or amniocentesis, should be limited to high-risk patients owing to the potential risks for procedure-related pregnancy loss.

Genome-wide sequencing tests for fetal diagnostics have also been employed and are expected to increase in popularity as the cost decreases and as new tools are developed. These tests include DNA sequencing methods, such as whole exome-sequencing and targeted clinical panels, which can further evaluate fetal structural anomalies first detected in an ultrasound (ISPD, 2018). This diagnostic sequencing method has been used for various fetal diagnostic measures including standard genetic testing and chromosomal microarray analysis. While cfDNA could theoretically be analyzed to screen for other genetic disorders beyond common aneuploidies, no professional guideline currently recommend expanded screening for additional genetic disorders (Glenn E Palomaki, 2022). Screening for these additional genetic conditions (other aneuploidies, microdeletions/microduplications, and single gene disorders) while increasingly commercially available, has not been recommended for routine use by leading medical societies and is considered “investigational” (Glenn E Palomaki, 2022).

Chromosomal microarray (CMA) testing refers to the use of comparative genomic hybridization (CGH) arrays to compare the DNA of a patient with a normal control (Aradhya et al., 2007). CMA is significantly more sensitive (10 to 100 kb) than traditional karyotyping (5 to 10 Mb), has a turnaround time of five days quicker than karyotyping (Robson et al., 2017), and provides an alternative to karyotyping when dividing cells are not available for analysis. This technique may be used for several different purposes, such as identifying a cause of pregnancy loss or identifying other aneuploid conditions, such as Down Syndrome (Reddy et al., 2012). This method of diagnostic prenatal sequencing is currently investigational because of limited data and is utilized most prominently in research settings or clinically on a case-by-case basis (ISPD, 2018).

Biochemical Markers in Maternal Serum

Many studies revealed that maternal age, fetal NT, maternal serum free β-human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein-A (PAPP-A) have been associated with aneuploidy. The "Quad screen," comprising alpha-fetoprotein (AFP), hCG, unconjugated estriol (E3), and inhibin-A, is the most efficient multiple-marker screening test in the second trimester. In addition, there are more options such as integrated, sequential testing, and cell-free DNA screening. Many studies are ongoing to reveal the most sensitive, specific and effective screening tools for use during the first trimester (Park et al., 2016).

To improve the accuracy of serum markers, ultrasound markers are used. NT refers to the fluid filled space measured on the dorsal aspect of the fetal neck. An enlarged NT (>3.0 mm/99th percentile of the crown-rump length) is independently associated with fetal aneuploidy and structural malformations (ACOG, 2016, 2020).

Screening studies of pregnant individuals reported an association between increased NT in the first trimester of pregnancy (10-13 weeks of gestation) and chromosomal defects, most commonly Down syndrome (trisomy 21) but also trisomy 18 and 13. NT could be done alone as a first-trimester screen or in combination with maternal serum markers, free beta subunit of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A). All three trisomies (chromosomes 13, 18, and 21) "are associated with increased maternal age, increased fetal NT and decreased PAPP-A, but in trisomy 21 serum free β-hCG is increased whereas in trisomies 18 and 13 free β-hCG is decreased" (Shiefa et al., 2013). Low β-hCG in the first trimester has also been associated with an increased risk of significant copy number variants on chromosomal microarray analyses (Bornstein et al., 2018).

Analytical Validity of Biochemical Markers

Screening for chromosomal abnormalities using biochemical markers include the first trimester combined test, triple test, quadruple test, sequential test, and integrated test. Except for the first trimester combined test, all others can provide screening results in the second trimester. In the first trimester combined test, the risk is calculated based on the ultrasonographic findings of NT and maternal serum levels of free β-hCG and PAPP-A. First-trimester screening not only allows early reassurance or early diagnosis of aneuploidy, but it also provides an option of earlier and safer termination of pregnancy in affected cases. Consequently, the first trimester combined test has become one of the most popular and useful screening strategies. Lee et al. (2013) conducted a 13-year study of 25,104 pregnant individuals using the first trimester Down syndrome screening. "The detection rates for trisomy 21, trisomy 18, Turner syndrome, and other chromosome anomalies were 87.5% (21/24), 69.2% (9/13), 81.8% (9/11), and 60% (18/30), respectively, with a false positive rate (FPR) of 5.4% (1353/25,026). Further evaluation of the detection rates for trisomy 21, by gestational age at 11, 12, and 13 weeks, were 92.3%, 87.5%, and 66.7%, respectively" (Lee et al., 2013).

For second-trimester screening for Down syndrome, the sensitivity and specificity of the triple test—co-testing AFP, unconjugated E3, and free β-hCG—are higher than screening with AFP alone. However, when the false-positive rate is fixed at 5% in order to compare the screening performance between the screening tools, the detection rate was found to be 66.8% to 77% with the triple test and 75.9% to 92% with the first trimester combined test. The sensitivity of the triple test was lower than the combined test (Baer et al., 2015).

The quadruple test, which uses the fourth marker, inhibin-A, in addition to the other three markers, has 7% higher sensitivity when applying a fixed 5% false-positive rate. A study conducted by Wald et al. (2003) revealed that when inhibin-A was added to the traditional triple marker test, a detection rate of 83% was achieved, which was 6% higher than the 77% detection rate found with the triple test. This result was similar to that produced with the first trimester combined test (Park et al., 2016).

Many studies, including the Serum, Urine and Ultrasound Screening Study (SURUSS) (Wald et al., 2003) and the First-and Second-Trimester Evaluation of Risk (FASTER) study (Malone et al., 2005) have offered evidence suggesting that first-trimester screening for Down syndrome with measurement of fetal NT and maternal serum markers is at least as accurate as alternative tests and may allow earlier confirmation or exclusion of Down syndrome. These studies evaluated several tests in parallel, including first-trimester testing with NT and maternal markers, the triple test, second-semester quadruple test and a combined first- and second-trimester test (both with and without NT), stepwise sequential testing (results given after first-trimester testing, move on to second-trimester testing), and integrated

screening (results given only after first and second-trimester testing). In a direct comparison of the first-trimester test to the triple test, the SURUSS study has shown that setting the false-positive rate at 5% resulted in an 83% detection rate, which was superior to what was historically expected of the triple test (Wald et al., 2003). SURUSS results were based on data from 47,053 pregnancies (101 with Down syndrome). The FASTER trial was conducted in the United States and was sponsored by the National Institutes of Health. The study enrolled 38,167 pregnant individuals and provided further evidence that first-trimester combined screening was effective, but it did not provide NT measurement alone; results showed that integrated first- and second-trimester screening provided higher detection rates. The SURUSS and FASTER studies also found that overall, first-trimester screening with NT alone is inferior to either first- or second-trimester combined screening. Additional testing may not be necessary in those few cases when NT is at least 4.0 mm due to the high likelihood of Down syndrome in these cases (Malone et al., 2005; Wald et al., 2009; Wald et al., 2003).

Studies have found a high rate of successful imaging of the fetal nasal bone and an association between absent nasal bone and the presence of Down syndrome in high-risk populations. However, there is insufficient evidence on the performance of fetal nasal bone assessment in average-risk populations. Of concern is the low performance of fetal nasal bone assessment in a subsample of the FASTER study conducted in a general population sample. Two studies conducted outside of the United States have found that, when added to a first-trimester screening program evaluating maternal serum markers and NT, fetal nasal bone assessment can result in a modest decrease in the false-positive rate. Several experts in the field are proposing that fetal nasal bone assessment be used as a second stage of screening to screen pregnant individuals found to be of borderline risk using maternal serum markers and NT. Considering the uncertainty of test performance in average-risk populations and the lack of standardization in the approach to incorporating this test into a first-trimester screening program, detection of fetal nasal bone is considered investigational (Wald et al., 2009).

Palomaki and colleagues demonstrate that hyperglycosylated hCG (h-hCG), also known as invasive trophoblast antigen (ITA), may be a promising screening marker for Down syndrome detection in the second trimester. In the study, serum samples of 45 Down syndrome cases and 238 unaffected control pregnancies between 14 to 20 weeks of gestation were collected and measured for h-hCG, along with other screening markers (Palomaki et al., 2004). As seen in the figure below, h-hCG, in combination with four other screening markers, increased the detection rate to 83% at a 5% false-positive rate from the 72% detection rate by the tripe test (Palomaki et al., 2004; QuestDiagnostics, 2019). In addition, "The median [h-hCG] in Down syndrome pregnancies was >3.00 multiples of the median, higher than that found for human chorionic gonadotropin (hCG)." The author recommends that "the highest screening performance for Down syndrome can be obtained by integrating first- and second-trimester serum and ultrasound markers into a single interpretation in the second trimester. This integrated test approach can detect 90% of Down syndrome pregnancies at a 3% false-positive rate" (Palomaki et al., 2004).

Cell-Free Fetal DNA from Maternal Serum

In 1997, researchers reported the identification of cell-free fetal DNA (cff-DNA) in the circulation of pregnant women (Lo et al., 1997). The fetal fraction of cff-DNA increases throughout gestation. cffDNA is usually detectable within six to seven weeks of gestation, with the earliest detection reported at 4.5 weeks. Therefore, it allows for non-invasive procedures to be performed much earlier in the pregnancy and eschews the need for standard biochemical and invasive screens. Moreover, given that cffDNA is

cleared from maternal circulation within hours following birth and removal of the placenta, cffDNA is specific to the ongoing pregnancy at the time of sampling (Shaw et al., 2020). At 10 weeks of gestation, the fetal fraction comprises at least 3 – 4% whereas it can account for up to 50% of the total cell-free DNA at term (Palomaki et al., 2018). Since then, laboratories have validated several different techniques for the use of cell-free DNA as a screening test for fetal aneuploidy, and these methods have been termed Non-Invasive Prenatal Screening or Testing (NIPS or NIPT).

Non-Invasive Prenatal Screening is a testing method which utilizes cell-free DNA from the plasma of pregnant individuals to screen for fetal aneuploidy. It is important to note that cell-free DNA screening does not assess risk of fetal anomalies, including neural tube defects or ventral wall defects (ACOG, 2015). NIPS methods only provide an estimate of whether the risk of aneuploidy is increased or decreased; NIPS does not provide a definitive diagnosis of aneuploidy. As with other aneuploidy screening tests, it is recommended that positive results of NIPS be followed by diagnostic testing such as traditional karyotyping of fetal cells obtained via chorionic villus sampling or amniocentesis (Gregg et al., 2016).

One cell-free fetal DNA detection method for NIPS, known as massively parallel sequencing (MPS), is a technique in which millions of pieces of maternal and fetal chromosomal material are sequenced and quantified. The MPS method is able to detect many types of aneuploidies, including those which are less commonly seen (Devers et al., 2013). MPS can detect common aneuploidies with both high sensitivity and high specificity for trisomies 13, 18, and 21. Bianchi et al. (2012) found the detection rate sensitivity for trisomy 21 to be 100%, the detection rate sensitivity for trisomy 18 to be 97.2%, and the detection rate sensitivity for trisomy 13 to be 78.6%; specificity was 100% for all three of the aforementioned trisomies.

Detection of aneuploidy using circulating cell-free fetal DNA can also be performed using selective analysis of specific loci only from the chromosomes of interest, as opposed to sequencing of all chromosomes performed in MPS. This directed analysis of cell-free fetal DNA has also been shown to have high sensitivity and high specificity for the common trisomies. Lee et al. (2019) utilized plasma from 1,055 pregnant individuals and found that NIPT with cell-free fetal DNA "showed 100% sensitivity and 99.9% specificity for trisomy 21, and 92.9% sensitivity and 100% specificity for trisomy 18, and 100% sensitivity and 99.9% specificity for trisomy 13."

The third approach to detect aneuploidy from cff-DNA is based on the amplification of single nucleotide polymorphisms (SNPs) on the chromosome of interest. In a study by Eiben et al. (2015), 2,942 patients underwent SNP-based non-invasive prenatal screening (NIPS) in which the source for cff-DNA was derived from placental cells. Sixty-five patients (2.2%) had positive non-invasive prenatal screening results for aneuploidy and further invasive testing confirmed aneuploidy in fifty-nine of those patients (90.8%). The remaining six patients were false positives due to a discrepancy between the genetic status of the fetus and placenta, a condition known as confined placental mosaicism (CPM). The fetal fraction was abnormally low (<8%) and indicative of fetal-placental discrepancies. Although a reliable screening method, the author suggests that SNP-based NIPS "cannot be used as a standalone test without ultrasound examination or invasive confirmation (Eiben et al., 2015)."

Despite the apparent advantages of NIPS over standard maternal serum screening in screening for common aneuploidies, there are limitations. "Reported Ifs [incidental findings] range from fetal or

maternal deletions and duplications or mosaic sex chromosome aneuploidy in the mother or fetus, presenting as aneuploidy risk on NIPS, to mosaicism and uniparental disomy to abnormal results because of the presence of cell-free DNA originating from an undiagnosed maternal tumor" (Westerfield et al., 2014). When ultrasound evaluation reveals fetal anomalies that may be consistent with one of those scenarios, invasive diagnostic testing with karyotyping or microarray may be more appropriate. NIPS also cannot distinguish the cause of aneuploidy, nor can it differentiate among the presence of an extra chromosome, a Robertsonian translocation, or high-level mosaicism. The determination of the type of aneuploidy is important for accurate counseling and future risk assessment (Neufeld-Kaiser et al., 2017; Strom et al., 2017; Westerfield et al., 2014). Also, some samples contain insufficient amounts of cell-free DNA, which is unknown until the test procedure has commenced. Early gestational age (<10 weeks) and high body mass index have been shown to be associated with reduced amounts of circulating cell-free fetal DNA. Additionally, NIPS for aneuploidy does not detect the presence of neural tube defects, which is included in traditional second trimester maternal serum screening. It has been suggested that the testing of maternal serum AFP in the second trimester should be offered to pregnant individuals who underwent first-trimester aneuploidy screenings (Palomaki et al., 2020).

And so, while promising on the screening front, research has yet to support NIPT's diagnostic prowess. NIPT platforms typically screen for common trisomies with or without sex chromosome anomalies, and therefore overlook most other chromosomal rearrangements (Al Toukhi et al., 2019; Shaw et al., 2020). Furthermore, the power of NIPT is limited by discordant—e.g., false positive and false negative—results, due to issues including vanishing twin syndrome, where a spontaneous early miscarriage may still release cffDNA and interfere with early NIPT results. Abnormal maternal cells mixing with normal fetal cells, producing mosaicism as aforementioned, has been reportedly repeatedly and therefore is an incidental cause of discordant results, suggesting that pregnant individuals with known malignancies should be dissuaded from NIPT (Bianchi et al., 2015; Shaw et al., 2020).

Extension of NIPT to sex chromosome aneuploidies and rare autosomal trisomies has also been explored, though its utility remains controversial. The increased variability in its use here is due in part to the sensitivity of NIPT to detect sex chromosome aneuploidies—e.g., Turner syndrome (45, X) and Klinefelter syndrome (45, XXY)—being lower than that of common trisomies. Moreover, as NIPT screens were originally limited in scope to identify trisomies 13, 18, and 21, the utility of NGS-based NIPT to also detect rare autosomal trisomies (RATs) has yet to be informed by the clinical community, and offers inspiration for future directions (Shaw et al., 2020).

Analytical Validity of Cell-free Fetal DNA Testing

A study by Palomaki and colleagues of 4664 pregnancies at high-risk for Down syndrome using the MPS method had a detection rate of 98.6% with a false-positive rate of only 0.20% (3/1471) (G. E. Palomaki et al., 2011). Results also identified MPS as a successful detection method for both trisomy 18 and trisomy 13. The authors state, "Among the 99.1% of samples interpreted (1,971/1,988), observed trisomy 18 and 13 detection rates were 100% (59/59) and 91.7% (11/12) at false-positive rates of 0.28% and 0.97%, respectively… Among high-risk pregnancies, sequencing circulating cell-free DNA detects nearly all cases of Down syndrome, trisomy 18, and trisomy 13, at a low false-positive rate. This can potentially reduce invasive diagnostic procedures and related fetal losses by 95%. Evidence supports clinical testing for these aneuploidies" (Palomaki et al., 2012).

Norton et al. (2015) reported near-perfect accuracy of detection for trisomy 21 (Down syndrome) with the use of cell-free DNA (cfDNA) (sensitivity, 100% [38 of 38 cases of trisomy 21]; false positive rate, 0.06% [9 false positives among 15,841 pregnant individuals]) in the Noninvasive Examination of Trisomy (NEXT) study. Norton and colleagues found that cfDNA testing for trisomy 21, as compared with standard screening, had a better global performance during the first trimester of pregnancy. However, they did not provide information about the 14 fetal chromosomal abnormalities in the 15,841 screened pregnancies, other than for trisomies 13, 18, and 21 (Norton et al., 2015).

In 2017, the Dutch Ministry of Health introduced a nationwide implementation study on NIPT as a first-tier strategy offered to all pregnant individuals in the TRIDENT-2 study. TRIDENT-2 was specific in its scope, as it excluded pregnancies with a vanishing or dischorionic twin, fetal ultrasound including a nuchal translucency greater than or equal to 3.5mm, or gestational age less than 11 weeks. Moreover, pregnant individuals with a history of being high-risk for the common trisomies and who have had an organ transplant were excluded as well, as were pregnant individuals with malignant neoplasia. Of all pregnancies that year, 73,239 (42%) opted for NIPT, it was found that though the number of common trisomies 13, 18, and 21 detected by NIPT was comparable to those of earlier studies, PPVs were higher than expected (53% PPV, 98%, 96%, respectively) with high sensitivities (100%, 91%, 98%, respectively), as confirmed by invasive prenatal testing or by postnatal bloodwork (van der Meij et al., 2019). However, the researchers do acknowledge potential limitations, namely not having presented data on sex chromosome aneuploidies and using different sequencing methods (e.g., NextSeq vs. HiSeq) and fetal fraction benchmarks for rejection across their three testing centers. However, despite issues to external validity, the authors conclude that "this study has confirmed that genome-wide NIPT is a reliable and robust screening test for the detection of fetal trisomies 21, 18, and 13" as they urge further research on screening for fetal pathology and adverse pregnancy outcomes (van der Meij et al., 2019).

As a secondary finding of the TRIDENT-2 study, NIPT may play a future role in raising suspicion of rare maternal malignancies. The TRIDENT-2 study enrolled 231,896 pregnant patients with NIPT results and showed incidental findings of malignancy-suspicious-NIPT in a small proportion of patients (Heesterbeek et al., 2022). The most common malignancies in women of reproductive age include breast, cervical, ovarian, and colorectal cancers; leukemia; Hodgkin and non-Hodgkin lymphoma; thyroid cancer; and melanoma. When tumor cfDNA is incidentally found in maternal blood, there are no current professional guidelines that address the clinical management of cfDNA results. While only (0.02%) of NIPT results in the Heesterbeek et. al study showed indications of a maternal malignancy, researchers pointed out this information has clinical significance and can prompt a diagnostic workup through magnetic resonance imaging and computed tomography to enable counseling and confirmative diagnosis in these rare instances (Heesterbeek et al., 2022).

Luo et al. (2021) aimed to explore the efficacy of using NIPT to predict sex chromosome aneuploidies (SCAs) in a 34,717-patient sample study in China. Of the clinical pregnancies examined, 229 (0.66%) were associated with sex chromosome aneuploidies, with 78 of the cases reporting positive for 45,X and 151 sex chromosome trisomies (47,XXX, 47,XXY, 47XYY). 193 of the 229 NIPT positive results acquiesced to confirmatory invasive prenatal diagnosis via karyotyping analysis of amniotic fluid and fluorescent *in situ* hybridization, and it was found that only 67 (34.7%) were true positives. The authors reported similarly low PPVs, with 23.07% for 45,X and 36%, 50%, and 27.27% for 47,XXX, 47,XXY, 47XYY, respectively. Given this performance of the NIPT, the authors concluded that "Confirmatory testing of abnormal results is recommended prenatally or after birth," insinuating the current impotency of NIPT (Luo et al., 2021).

A two-year longitudinal study which utilized 11,414 material blood samples for NIPT found that "The overall sensitivity of NIPT was 98.90, 100.00, 100.00, 90.91, 100.00, 100.00 and 100.00%, and specificities were 99.96, 99.97, 99.99, 99.96, 99.98, 100.00 and 99.99% for detecting T21, T18, T13, XO, XXX, XYY and XXY, respectively" (Garshasbi et al., 2019). Hence, this testing shows excellent potential in the detection of fetal aneuploidies.

A study out of the Illumina laboratory (formerly Verinata) compared NIPS to standard maternal serum screening in pregnant women at average risk for fetal aneuploidy. Their report included data of 5974 samples tested for trisomies 13, 18, and 21 as well as monosomy X. Aneuploidy was detected in 4.8% of samples with only 0.2% putative false-positives and 0.08% false-negatives; however, 2.8% of cases had indefinite results for a single chromosome (Futch et al., 2013). Illumina more recently reported a more extensive study consisting of 85,298 clinical cases. "Aneuploidy was detected or suspected in 2142 (2.5%) samples. For aneuploidy detected cases with known clinical outcomes, the overall positive predictive value (PPV) was 83.5% (608/728); observed PPVs for trisomies 21, 18, and 13 ranged from 50.0 to 92.8%" (Taneja et al., 2016).

A retrospective study by Wu et al. (2020) compared positive non-invasive prenatal screening (NIPS) results for aneuploidy to standard diagnostic tests such as traditional karyotyping and chromosomal microarray analysis (CMA). The study enrolled 551 pregnant individuals who screened positive for trisomy 13, trisomy 18, trisomy 21, and other sex chromosomal aneuploidies. Samples were obtained from either amniotic fluid or fetal cord blood and subsequent karyotyping or CMA confirmed a total of 256 out of 551 cases (46.4%) to possess chromosomal abnormalities concordant or partially concordant with NIPT results. Placental biopsies were obtained to assess the etiology of NIPS false positives and confined placental mosaicism (CPM) was found in 60% of the biopsies. The authors also reported that pregnant individuals with advanced maternal age (>35 years) had the highest positive predictive value (PPV) for trisomy 21 (87.8%), trisomy 18 (59.3%), and trisomy 13 (37.5%), while the PPV was significantly lower for pregnant individuals with young maternal age (<34 years) for trisomy 21 (71.9%), trisomy 18 (0%), and trisomy 13 (16.7%). This suggests that NIPS performs better in predicting aneuploidies for pregnancies with advanced maternal age than for pregnancies with young maternal age. However, the author notes that the PPVs showed "no significant upward trend when compared based on specific age categories (an interval of 5 years), which suggested that NIPT-positive result deserves equal attention from both providers and patients regardless of maternal age" (Wu et al., 2020).

Table 1. Down Syndrome Detection Rates (DRs) Obtained from Various Screening Tests[12]

Test Name	Alias	Markers Included	DR, %[a]
Triple Screen	Maternal Serum Screen 3	Age, AFP, hCG, uE3	72
Quad Screen	Maternal Serum Screen 4	Age, AFP, hCG, uE3, DIA	79
Penta Screen	Maternal Serum Screen 5	Age, AFP, hCG, uE3, DIA, h-hCG	83

[a] Detection rate at a 5% false-positive rate.

Dar et al. (2022) investigated the performance of cell-free DNA screening against a genetic confirmation of results with the goal of analyzing test performance and test failure (no-call rates). A total of 20,194 pregnant individuals were enrolled with a median gestational pregnancy of 12.6 weeks. The results of the genetic test were confirmed for 17,851 cases (88.4%). Among these were 13,043 low-risk and 4808

high-risk cases for aneuploidy. A total of 133 trisomies were diagnosed. These were composed of 100 (trisomy 21), 18 (trisomy 18) and 15 (trisomy 13). The positive rate of cfDNA screens was lower in the low-risk group as compared to the high-risk cohort (0.27% versus 2.2% P<.0001). Sensitivity and specificity were comparable between the two risk groups. The PPV for the low- and high-risk groups was 85.7% versus 97.5%, respectively. There were also 602 individuals who had a "no-call" outcome post-draw and 287 (1.61%) after a second draw. The authors concluded that "in women at a low risk for aneuploidy, single-nucleotide polymorphism-based cell-free DNA has high sensitivity and specificity, positive predictive value of 85.7% for trisomy 21 and 74.3% for the 3 common trisomies." They also noted that "patients who receive a no-call result are at an increased risk of aneuploidy and require additional investigation"(Dar et al., 2022).

Proprietary Testing

Several methods for detection of fetal aneuploidy by analysis of circulating cell-free fetal DNA are commercially available. All have been validated in pregnancies deemed to be at high risk for aneuploidy. Evaluation of this technology for use in low- or average-risk pregnancies is ongoing.

Current commercially available laboratory-developed non-invasive prenatal tests for aneuploidy include: the MaterniT21™ Plus Test(Integrated Genetics/LabCorp) (LabCorp, 2022), the verifi™ Prenatal Test and verifi™ Plus Prenatal Test (Illumina) (Illumina, 2022), the Harmony Prenatal Test™ (Roche) (Roche, 2022), the Panorama test (Natera, available from several reference laboratories) (Natera, 2022), QNatal® Advanced test (QuestDiagnostics, 2022), Prequel test (Myriad, 2022), CentoNIPT® test (Centogene, 2022), ClariTest™ Core (GenPath, 2022), IONA® test and Sage™ prenatal screen (YourgeneHealth, 2022), Invitae NIPS test (Invitae, 2022), and Clarigo test (AgilentTechnologies, 2022). Other examples include, but are not limited to, the VERACITY® test out of NIPD Genetics (NIPD, 2022), the Vanadis® NIPT system (PerkinElmer, 2022), the NIFTY® Test and NIFTY® Test Pro (BGI, 2022), and the informaSeq® Prenatal Test (Genetics, 2016).

Regarding serum screening options for common birth defects, Integrated Genetics, a LabCorp Specialty Testing Group, names the Afp4®, which screens for Down syndrome, trisomy 18, and open neural tube defects in the second trimester, and SerumIntegratedScreen®, which screens for the same, but combines results from both the first and second trimesters.

Table 1. Down Syndrome Detection Rates (DRs) Obtained from Various Screening Tests[12]

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[a] Detection rate at a 5% false-positive rate.

Other tests mentioned include the FirstScreen®, IntegratedScreen®, and SequentialScreen®, the latter of which boasts that "Part 1 [between 10th and 14th weeks of pregnancy] leads to the detection of approximately 70% of Down syndrome cases and 80% of trisomy 18 cases, and Part 2 [between 15th and

22nd weeks of pregnancy] leads to detection of approximately 90% of Down syndrome cases, 90% of trisomy 18 cases and 80% of open neural tube defects" (LabCorp, 2021).

The Luna Prenatal test is a proprietary prenatal test that purports to be the "only noninvasive prenatal genetic test that offers reliable detection of deletions and duplications down to a resolution of 1.5 and 2.0 megabases (Mb), respectively, no false positives stemming from the maternal genotype, and less interference from high BMI" (Luna, 2022a). A white paper notes that an important benefit of the Luna Prenatal test is the ability to analyze a single fetal cell (trophoblast), providing access to only fetal DNA.

In an analytical validation overview of the Luna prenatal test, two groups of pregnant individuals (one group composed of 59 high-risk individuals who received CVS or amniocentesis) and a second group (158 low-risk individuals who had no known diagnostic testing) were recruited. A third set of test samples was analyzed for comparison composed of "seven Coriell cell lines harboring known chromosome abnormalities" (Luna, 2022b). Single cells (enriched from maternal whole blood) were processed individually to access pure fetal or cell line DNA. Subsequently, the DNA from each individual cell was "sequenced by low-pass massively parallel sequencing technology to generate a genome-wide copy number profile, which was evaluated for genome-wide copy number changes larger than 1.5 Mb"(Luna, 2022b). The first group of 59 samples had 11 samples excluded. Of the remaining 48 pregnancies (49 fetuses), results indicated an accuracy of 100% (92.75-100% confidence interval), specificity 100% (54.07-100% confidence interval), sensitivity of 100% (91.78-100% confidence interval), PPV of 100%, and NPV of 100% in detecting chromosomal abnormalities (complete agreement between CVS/amniocentesis and Luna); they also noted a plan to increase the sample size to 200 for a larger data set as this was a limited study.

For the second test group, a report was issued for 91.1% of cases, with 8.9% receiving a result of "no scorable cells." In the second group validation study, they found "complete intra-case concordance for 404 cells from 118 cases" (Luna, 2022b). The third set used human cultured cells (lymphoblast or fibroblast) with known aneuploidy or deletion. From the results: "out of a total of 148 scorable cells, the known abnormality was detected and called by the NxC software in every cell except one. Thus, the analytic sensitivity and specificity were 100% with the expected finding being reported in every cell" (Luna, 2022b).

The test makers also note: "We do not claim that the Luna Prenatal Test is diagnostic for aneuploidy, because there are recommendations from professional organizations that a final diagnosis should not rest on testing of trophoblasts alone" (Luna, 2022b).

V. Guidelines and Recommendations

The American College of Obstetrics and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM)

ACOG and SMFM offered Recommendations for Clinical Management Guidelines for Obstetricians and Gynecologists on Screening for Aneuploidy.

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- "Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to all pregnant women regardless of maternal age or risk of chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.

- If screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously.

- Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing.

- All patients should be offered a second-trimester ultrasound for fetal structural defects, since these may occur with or without fetal aneuploidy; ideally this is performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein).

- Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results.

- Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected. The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings become such as fetal anomalies identified on ultrasound examination become evident.

- Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.

- If an enlarged nuchal translucency or an anomaly is identified on ultrasound examination, the patient should be offered genetic counseling and diagnostic testing for genetic conditions as well as a comprehensive ultrasound evaluation including detailed ultrasonography at 18–22 weeks of gestation to assess for structural abnormalities.

- Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing" (ACOG, 2018, 2020).

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- "The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test. However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.

- In clinical situations of an isolated soft ultrasonographic marker (such as echogenic cardiac focus, choroid plexus cyst, pyelectasis, short humerus or femur length) where aneuploidy screening has not been performed, the patient should be counseled regarding the risk of aneuploidy associated with the finding and cell-free DNA, quad screen testing, or amniocentesis should be offered. If aneuploidy testing is performed and is low risk, then no further risk assessment is needed. If more than one marker is identified, then genetic counseling, maternal–fetal medicine consultation, or both are recommended.

- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be incorporated into pretest counseling for patients with multiple gestations.

- Cell-free DNA screening can be performed in twin pregnancies. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13" (ACOG, 2020).

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- "The use of multiple serum screening approaches performed independently (e.g., a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver contradictory risk estimates.

- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.

- Patients with unusual or multiple aneuploidies detected by cell-free DNA should be referred for genetic counseling and maternal–fetal medicine consultation" (ACOG, 2020).

ACOG also comments on the specific types of screening, which include triple, quadruple (quad) and "penta" screens. These screens may be performed in the first trimester (10-14 weeks gestation) or second trimester (15-22 weeks). Triple screens measure serum hCG [human chorionic gonadotropin], AFP [alphafetoprotein], and uE3 [unconjugated estriol], while the quad screen includes DIA [dimeric inhibin A] with the three previously mentioned markers. Some laboratories have been noted to offer a "penta" screen, which includes hyperglycosylated hCG along with the four analytes of the quad screen,

Prenatal Screening for Fetal Aneuploidy, continued

but ACOG states that "its performance has not been evaluated rigorously in prospective studies". ACOG discusses several testing algorithms in the include, which are summarized in the table below:

Screening Approach	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate (DR) for Trisomy 21 (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
Cell-free DNA†	9–10 to term	99	2–4% Includes inability to obtain results, which is associated with increased risk†	1. Highest DR 2. Can be performed at any gestational age after 9–10 weeks 3. Lowest false-positive rate	Results may reflect underlying maternal aneuploidy or maternal disease	Several molecular methods
First trimester‡	10–13 6/7§	82–87‖	5	1. Early screening 2. Single time point test	Lower DR than tests with first and second trimester component NT required	NT+PAPP-A, free beta hCG, +/− AFP¶
Quad screen‡	15–22	81	5	1. Single time point test 2. No specialized US required	Lower DR than first trimester and first and second trimester combined tests	hCG, AFP, uE3, DIA
Integrated‡	10–13 6/7§, then 15–22	96	5	High DR	Two samples needed No first-trimester results NT required	NT+PAPP-A, then quad screen
Serum integrated‡	10–13 6/7§, then 15–22	88	5	1. DR compares favorably with first-trimester screening 2. No specialized US required	Two samples needed No first-trimester results	PAPP-A + quad screen
Sequential#: stepwise	10–13 6/7§, then 15–22	95	5	1. First-trimester results provided 2. Comparable performance to integrated, but FTS results provided	Two samples needed NT required	NT+ free beta hCG + PAPP-A, +/− AFP¶, then quad screen
Contingent screening**		88–94	5	First-trimester test result: Positive: diagnostic test or cell-free DNA offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed NT required	NT+hCG+ PAPP-A, +/− AFP¶, then quad screen

Finally, ACOG notes that other trisomies, such as trisomies 16 or 22, can be tested for. However, ACOG recommends against screening for these two aneuploidies due to lack of validated data (ACOG, 2020).

Society for Maternal-Fetal Medicine (SMFM) through Choosing Wisely

SMFM submitted fifteen short recommendations regarding maternal and fetal medicine through Choosing Wisely. The relevant recommendation is as follows:

"Don't order serum aneuploidy screening after cfDNA [cell-free DNA] aneuploidy screening has already been performed" (SMFM, 2019).

The National Society of Genetic Counselors (NSGC)

The NSGC issued a position statement that supports noninvasive prenatal screening as an option for pregnancies considered high risk for trisomy 13, 18 or 21. "The National Society of Genetic Counselors currently supports Noninvasive Prenatal Testing/Noninvasive Prenatal Diagnosis (NIPT/NIPD) as an option for patients whose pregnancies are considered to be at an increased risk for certain chromosome abnormalities. NSGC urges that NIPT/NIPD only be offered in the context of informed consent, education, and counseling by a qualified provider, such as a certified genetic counselor. Patients whose NIPT/NIPD results are abnormal, or who have other factors suggestive of a chromosome abnormality, should receive genetic counseling and be given the option of standard confirmatory diagnostic testing" (Devers et al., 2013).

The NSGC expounded upon their recommendations for prenatal screening and diagnostic testing for chromosomal aneuploidy in a set of practice guidelines. For all patients, it is recommended that "Providers should offer the options of maternal serum screening (MSS) and diagnostic testing for chromosome aneuploidy to every patient", provided that the providers themselves are made aware of factors that may impact their patients' options and that the patients are made aware of the costs and benefits of such options. However, "An ultrasound to assess the fetal anatomy is suggested at approximately 18w0d-20w0d gestation for all patients regardless of whether or not they choose to have screening or diagnostic testing" (Wilson et al., 2013).

The NSGC also presented the following recommendations for low-risk patients less than 14 weeks of gestation:

- "For patients who may consider CVS [chorionic villi screening] or amniocentesis, *stepwise sequential screening or combined first trimester screening* should be considered".
- "If CVS is not an option, *integrated screening* may be considered in order to maximize detection rates."
- "If a patient completes combined first trimester screening, a separate second trimester MSS for chromosome aneuploidy is *NOT* indicated. Screening for chromosome aneuploidy in the second trimester in patients who present prior to 14 weeks should *ONLY* be performed as a part of integrated, serum integrated, stepwise sequential, or contingency screening."
- "Patients who have an increased NT [nuchal translucency] (≥ 95th % or ≥ 3.0mm) should be offered diagnostic testing by either CVS or amniocentesis. A referral for a fetal echocardiogram should also be considered if the NT ≥3.5mm."
- "Early amniocentesis (prior to 15 weeks of gestation) is *not* recommended due to the increased risks for pregnancy loss, clubfoot, and fluid leakage. CVS should be offered as the diagnostic testing option for chromosome aneuploidy in the first trimester."

For low-risk patients after 14 weeks of gestation, they recommend the following:

- "Patients who desire MSS but did not have MSS in the first trimester should be offered a *quad* or *penta* screen rather than a triple screen due to the increased detection rates.
- Amniocentesis should be offered as the diagnostic testing option for chromosome aneuploidy for patients after 15 weeks of gestation."

The NSGC also recommend for those patients at increased risk for chromosome aneuploidy that if the patient presents prior to 14 weeks gestation, "CVS and amniocentesis should both be offered as diagnostic testing options for chromosome aneuploidy", whereas if the patient presents after 14 weeks gestation, "amniocentesis should be offered as the diagnostic testing option for chromosome aneuploidy." Lastly, the NSGC reiterated that patients may be offered NIPT (non-invasive prenatal testing) should they desire screening information (Wilson et al., 2013).

The International Society for Prenatal Diagnosis (ISPD)

The ISPD recognizes that massively parallel sequencing for detection of Down syndrome can be "helpful" for women with high-risk pregnancies, when "suitable genetic counseling" is provided (Benn et al., 2013).

The International Society for Prenatal Diagnosis released a statement regarding cell free DNA screening for Down syndrome in multifetal pregnancies in 2020. The following recommendations were made:

- "The use of first trimester cfDNA screening for the common autosomal trisomies is appropriate for twin pregnancies due to sufficient evidence showing high detection and low false positive rates with high predictive values.

- The finding of an increased risk on a cfDNA screening test in multiple pregnancies should be followed by counseling and an offer of diagnostic testing to confirm results…CVS and amniocentesis procedures in multiple pregnancies are reliable and safe when performed by a provider experienced in these situations; subsequent diagnostic tests are highly reliable… Maternal age and nuchal translucency (with or without biochemistry) detects up to 80% of Down syndrome at a 5% false positive rate in twin pregnancies.

- It is preferable for laboratories performing cfDNA testing in multifetal pregnancies to take evidence of zygosity (e.g., chorionicity, sex of the fetuses, embryo transfer history) for the interpretation of both test results and fetal fractions…Interpretation of the cfDNA test results could differ depending on test methodology, fetal fraction and chorionicity/zygosity…Fetal fractions are higher in twin pregnancies, but lower for individual fetuses when compared to singletons. Fetal fractions are correlated between dizygous twins but can still vary two-fold.

- cfDNA based screening for common trisomies in twins provides higher positive predictive values among twin pregnancies compared with traditional serum and nuchal translucency based screening in twins, but are associated with test failures…When a cfDNA test failure occurs consider ultrasound and diagnostic testing. If there is sufficient time, a second sample draw may also be considered.

- Screening options for triplet pregnancies are lacking and cfDNA may be a potential option. However, diagnostic testing should always be offered and the limitations of screening tests stressed" (Glenn E. Palomaki et al., 2020).

The American College of Medical Genetics and Genomics (ACMG)

The ACMG notes that "Pretest information should be provided … to ensure patients make informed decisions. Aneuploidy screening is not a routine prenatal test; it is acceptable for patients to decline screening." The ACMG also cautions that "All reports should clearly state that NIPS is a screening test and not diagnostic," and that results be presented in a clear and easily understandable fashion. ACMG guidelines state that "in pregnancies with multiple gestations and/or donor oocytes, testing laboratories should be contacted regarding the validity of NIPS before it is offered to the patient as a screening option" (Gregg et al., 2016).

Practice Committee and Genetic Counseling Professional Group (GCPG) of the American Society for Reproductive Medicine

The American Society of Reproductive Medicine notes that screening tests (maternal serum biochemical screening, nuchal translucency and fetal anatomy scan, and noninvasive prenatal screening with cell-free DNA) cannot diagnose chromosomal aneuploidy. "In some cases, ultrasound and biochemical analytes may help identify congenital anomalies that may be associated with an aneuploid pregnancy; however, many aneuploidies (and mosaic aneuploidies in particular) do not result in visible ultrasound anomalies or skewed biochemical analytes and may be easily missed." Cell-free DNA testing "may test for a select number of full and partial aneuploidies, or all aneuploidies within a specified chromosomal resolution, depending on the specific test used by the laboratory. If the chromosome or chromosomal segment of interest is in fact able to be assessed by the assay used, an aneuploidy may be detected. However, it is important to recognize that NIPT is not designed for the detection of mosaicism and may result in false-negative results. False-positive results may also occur because NIPT analyzes placental (and not fetal) DNA" (ASRM, 2020).

VI. Applicable State and Federal Regulations

Food and Drug Administration

In April 2021, the FDA released a statement titled, "Genetic Non-Invasive Prenatal Screening Tests May Have False Results: FDA Safety Communication," that detailed safety concerns regarding the use of NIPS tests outside of standard screening purposes. Specifically, they reinforced that NIPS tests are laboratory developed tests not regulated by the FDA and that the use of NIPS should be confined to screenings, rather than diagnoses. "The accuracy and performance of NIPS tests have not been evaluated by the FDA and these tests can give false results, such as reporting a genetic abnormality when the fetus does not actually have one. NIPS tests are screening tests, which means the NIPS test may only tell you the risk of the fetus having certain genetic abnormalities. They are not diagnostic tests, which are generally used to more definitively confirm or rule out a suspected genetic abnormality." Additionally, "The FDA recommends that patients discuss the benefits and risks of NIPS tests with a genetic counselor or other health care provider before deciding to get these tests. Patients should also discuss the results of NIPS tests with a genetic counselor or other health care provider before making any decisions about their

pregnancy. Health care providers should be aware of the risks and limitations of using these screening tests and should not use the results from these tests alone to diagnose chromosomal (genetic) abnormalities or disorders" (FDA, 2022).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81508	Fetal congenital abnormalities, biochemical assays of two proteins (PAPP-A, hCG [any form]), utilizing maternal serum, algorithm reported as a risk score
81509	Fetal congenital abnormalities, biochemical assays of three proteins (PAPP-A, hCG [any form], DIA), utilizing maternal serum, algorithm reported as a risk score
81510	Fetal congenital abnormalities, biochemical assays of three analytes (AFP, uE3, hCG [any form]), utilizing maternal serum, algorithm reported as a risk score
81511	Fetal congenital abnormalities, biochemical assays of four analytes (AFP, uE3, hCG [any form], DIA) utilizing maternal serum, algorithm reported as a risk score (may include additional results from previous biochemical testing)
81512	Fetal congenital abnormalities, biochemical assays of five analytes (AFP, uE3, total hCG, hyperglycosylated hCG, DIA) utilizing maternal serum, algorithm reported as a risk score
82105	Alpha-fetoprotein (AFP); serum
82106	Alpha-fetoprotein (AFP); amniotic fluid
82677	Estriol
84163	Pregnancy-associated plasma protein-A (PAPP-A)
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
86336	Inhibin A
88235	Tissue culture for non-neoplastic disorders; amniotic fluid or chorionic villus cells
88267	Chromosome analysis, amniotic fluid or chorionic villus, count 15 cells, 1 karyotype, with banding
88269	Chromosome analysis, in situ for amniotic fluid cells, count cells from 6-12 colonies, 1 karyotype, with banding
88271	Molecular cytogenetics; DNA probe, each (e.g., FISH)
88280	Chromosome analysis; additional karyotypes, each study
88285	Chromosome analysis; additional cells counted, each study

VIII. Evidence-based Scientific References

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Prescription Medication and Illicit Drug Testing in the Outpatient Setting

Policy #: AHS – T2015	**Prior Policy Name & Number (as applicable):** • AHS-T2015- Opioids Testing in Pain Management and Substance Abuse • AHS-T2015-Toxicology
Implementation Date: 9/15/21	**Date of Last Revision:** 4/27/22 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Abuse of both prescription and illicit drugs is extremely common. Drugs of abuse (DOA) may be defined as "a drug, chemical, or plant product that is known to be misused for recreational purposes," which can include drugs such as pain relievers that have legitimate prescriptions. Drug tests may be performed for a variety of reasons, such as compliance with treatment program or medical regimen. Numerous biological substances, such as blood, hair, or saliva may be tested, but urine is the most commonly tested biological substance in drug tests (Hoffman, 2021).

This policy addresses clinical toxicology in the outpatient setting and does not address forensic testing or therapeutic drug monitoring (TDM). Forensic drug testing is used for legal proceedings and requires secondary confirmatory testing (J. Jones, 2016). TDM "involves sampling of plasma or serum drug levels to determine optimal drug dosing" (Eaton & Lyman, 2019).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCD) for Medicare] for a particular member, then the government policy will be used to make the determination.

This policy concerns only coverage criteria and does not describe or define the legal responsibility of providers. Providers should refer to state and federal laws for such guidance.

This policy does not address the use of drug testing in the following circumstances:

A. State, federally regulated, and legally mandated drug testing (i.e., court-ordered drug screening, forensic examinations).

B. Non-forensic testing for commercial driver's licensing or any other job-related testing (i.e., as a prerequisite for employment or as a means for continuation of employment).

C. As a component of routine physical/medical examination.

D. As a component of care rendered in an urgent/emergency situation.

E. As a routine component of a behavioral health assessment.

Presumptive Drug Screening using Urine Samples

1. Presumptive drug screening using urine samples (qualitative, semi-quantitative or quantitative) **MEETS COVERAGE CRITERIA** in any of the following situations:

    a. To assess an individual being treated for chronic, non-cancer pain when clinical evaluation of the patient (history/signs/symptoms) suggests the use of non-prescribed medications or illegal substances:

        i. Prior to initiating chronic opioid pain therapy in chronic non-cancer pain to determine if the individual has been exposed to controlled substances or potentially confounding illicit drugs.

        ii. To verify an individual's compliance with treatment or identify undisclosed drug abuse as part of routine monitoring for individuals who are receiving treatment for non-cancer chronic pain with prescription opioid pain medication. The random testing interval and drugs selected for testing should be based on the individual's history, condition and treatment, as documented in the medical record.

            a) Monitoring of low risk (as defined by a risk assessment tool) individuals on chronic opioid therapy, up to one (1) time per year after initiation of therapy.
            b) Monitoring of moderate risk (as defined by a risk assessment tool) individuals on chronic opioid therapy, up to two (2) times per year after initiation of therapy.
            c) Monitoring of high risk (as defined by a risk assessment tool) individuals on chronic opioid therapy, up to four (4) times per year after initiation of therapy.
            d) For individuals with aberrant behavior (lost prescriptions, multiple requests for early refills, and opioids from multiple providers, unauthorized dose escalation, apparent intoxication, etc.) testing at the time of visit meets coverage criteria.

b. In pregnant individuals at high-risk for substance abuse in whom the suspicion of drug use exists based on the answers to substance abuse screening questions or as indicated by information from the prescription drug monitoring program (PDMP), as documented in the medical record.

c. In newborns when there is a history of maternal substance abuse or agitated/altered mental status in the birthing parent.

d. In candidates for organ transplant who have a history of substance abuse (to demonstrate abstinence prior to transplant).

e. In individuals with a suspicion of or a diagnosis of mental illness (e.g., anxiety disorders, schizophrenia, major depressive disorder, mood disorders, suicidal ideations, substance abuse disorder)

f. In individuals with attention-deficit hyperactivity and disruptive behavior disorders

g. In cancer patients on opioid pain medication

h. In individuals with epilepsy

i. For the management and compliance monitoring of an individual under treatment for substance abuse or dependence at the following frequency after baseline at initial evaluation and must be documented in the patient's medical record:

   i. For patients with 0 to 90 consecutive days of abstinence, random qualitative drug testing at a frequency of 1 to 2 per week.

   ii. For patients with > 90 consecutive days of abstinence, random qualitative drug testing at a frequency of 1 to 3 per month.

j. In individuals where substance abuse is in the differential diagnosis of the presenting condition.

Definitive Drug Testing

2. Confirmatory/definitive qualitative or quantitative drug testing (up to seven drug classes) **MEETS COVERAGE CRITERIA** when laboratory-based definitive drug testing is specifically requested, and the rationale documented by the patient's treating physician and any of the following conditions is met:

   a. The result of the presumptive drug screen is different than that suggested by the patient's medical history, clinical presentation or patient's own statement. (e.g., test was negative for prescribed medications, test was positive for prescription drug with abuse potential, which was not prescribed, test was positive for an illegal drug).

      i. The test was negative for prescribed medications, or

      ii. Positive for a prescription drug with abuse potential which was not prescribed, or

      iii. Positive for an illegal drug

   b. For diagnosing and monitoring individuals with substance use disorder or dependence, when accurate and reliable results are necessary for treatment decisions.

i. Individuals with 0 to 30 consecutive days of abstinence, random definitive drug testing at a frequency of not to exceed 1 per week.

ii. Individuals with 31 to 90 consecutive days of abstinence, random definitive drug testing at a frequency of 1 to 3 per month. No more than 3 definitive drug tests in one month will be allowed.

iii. Individuals with greater than 90 consecutive days of abstinence, definitive drug testing at a frequency of 1 to 3 every three months. No more than 3 definitive drug tests in a 3-month period will be allowed.

c. For monitoring of individuals on opioid therapy (to ensure adherence to the therapeutic plan, for treatment planning, and for detection of other, non-prescribed opioids).

d. A presumptive test does not exist or does not adequately detect the specific drug or metabolite to be tested (e.g., specific drugs within the amphetamine, barbiturate, benzodiazepine, tricyclic antidepressants, and opiate/opioid drug classes as well as synthetic/analog or "designer" drugs).

e. To definitively identify specific drugs in a large family of drugs

f. To identify drugs when a definitive concentration of a drug is needed to guide management

3. When laboratory-based definitive drug testing is requested for larger than seven drug classes panels confirmatory/definitive qualitative or quantitative drug testing **DOES NOT MEET COVERAGE CRITERIA**.

4. Confirmatory/definitive qualitative or quantitative or presumptive (qualitative, semi-quantitative or quantitative) drug testing using proprietary tests (e.g., CareView360) **DOES NOT MEET COVERAGE CRITERIA**.

Specimen Validity Testing

5. Specimen validity testing (e.g., urine specific gravity, urine creatinine, pH, urine oxidant level, and genetic identity testing [e.g., NextGen Precision Testing, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Documentation Requirements

The patient's medical record must contain documentation that fully supports the medical necessity for drug testing. This documentation includes, but is not limited to, relevant medical history, physical examination, and results of pertinent diagnostic tests or procedures.

Reimbursement

The following IS REIMBURSED (see complete Coverage Criteria in the relevant topics in Section III above) for:

1. **Presumptive drug screening** based upon appropriate clinical criteria (qualitative, semi-quantitative or quantitative);
2. **Definitive drug testing** (qualitative or quantitative) for up to seven drug classes when the presumptive drug screening meets one of the following criteria:
   a. The test was negative for prescribed medications, or
   b. Positive for a prescription drug with abuse potential which was not prescribed, or
   c. Positive for an illegal drug, or
   d. A presumptive test does not exist or does not adequately detect the specific drug or metabolite to be tested
3. Blood specimens in patients with anuric Chronic Renal Failure

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The following IS NOT REIMBURSED:

1. Any AMA definitive drug class codes and CPT 80307
2. Same-day testing of the same drug or metabolites from two different samples (e.g. both a blood and a urine specimen) by either presumptive or definitive analyses
3. Blanket orders or routine standing orders for all patients in the physician's practice

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Only urine or oral fluid specimens will be covered, except blood specimen will be covered for patients with anuric Chronic Renal Failure.

Confirmatory/definitive testing should be supported by documentation of rationale in the patient's medical record.

More than one presumptive test result per patient per date of service regardless of the number of billing providers **IS NOT REIMBURSED**:

1. It is not reasonable or necessary for a provider to perform qualitative point-of-care testing and also order presumptive testing from a reference laboratory on the same specimen.
2. It is not reasonable or necessary for a provider to perform presumptive immunoassay testing and also order presumptive immunoassay testing from a reference laboratory with or without reflex testing on the same specimen.

III. Scientific Background

According to the National Center for Drug Abuse Statistics, as many as 31.9 million Americans 12 or older used an illicit drug in the last 30 days, which corresponds to 11.7% of Americans overall and 39% for young adults from 18 to 25. 10.1 million misused opioids in the previous year, with 9.7 million misusing prescription pain relievers. Approximately 9.5 million adults had a concurrent mental illness and substance abuse disorder in the previous year (National Center for Drug Abuse Statistics, 2022) . A drug of abuse (DOA) may be defined as "a drug, chemical, or plant product that is known to be misused for recreational purposes," which can include drugs, such as pain relievers, that have legitimate prescriptions. Drug testing may be performed for several reasons. For example, patients in areas including pain management, substance abuse treatment, and psychiatric treatment have a higher propensity for substance abuse and must be monitored as such (Hoffman, 2022).

DOA screening varies in composition between countries. In the U.S., typical DOA screening tests encompass amphetamine, cocaine, marijuana/tetrahydrocannabinol (THC), opioids, and phencyclidine (PCP) as included in the United States' Drug-Free Workplace Act of 1988; these DOA are often referred as the SAMHSA 5, named after the Substance Abuse and Mental Health Services Administration (Hoffman, 2021; Phan et al., 2012). Although the drug trends have changed dramatically since 1988, these five have remained on the basic drug screen used across the U.S. The U.S. Department of Defense (DOD) removed PCP from its routine screening but added benzodiazepines, amphetamine derivatives, common barbiturates, synthetic and semisynthetic opioids, lysergic acid diethylamide (LSD), and synthetic cannabinoids. Other countries or regions, such as Australia and the European Union, also include testing for benzodiazepines and wider range of opioids (Hoffman, 2022). The American Society of Addiction Medicine (ASAM) recommends drug-testing panels based on "the patient's drug of choice, prescribed medications, and drugs commonly used in the patient's geographic location and peer group" rather than relying on the SAMHSA 5 (ASAM, 2017).

The testing performed could be qualitative, semi-quantitative or quantitative, presumptive or definitive. Qualitative refers to testing for the presence of a given analyte, semi-quantitative reports if the analyte is present above or below a certain threshold, and quantitative reports the exact amount of an analyte. Presumptive drug testing is used to identify use or non-use of a drug or a drug class, but this type of testing cannot distinguish between structural isomers. Definitive drug testing usually refers to a more definitive methodology, such as mass spectrometry or chromatography, because these methods can identify use or non-use of a specific drug and/or its associated metabolites. Both types of drug testing can be either quantitative or qualitative (P. J. Jannetto & Langman, 2018). The frequency of testing is usually determined by the providers; testing may be random or scheduled depending on the provider's objectives (Becker & Starrels, 2022).

Urine drug tests are the most common method of drug testing for several reasons. Unlike blood or saliva, the window of detection of most drugs is longer in urine; moreover, urine tests are inexpensive, noninvasive, and convenient to use while still maintaining acceptable statistical validity. Salivary testing can provide a higher rate of false-negative results, especially for individuals who smoke. Urine may provide more objective assessment of drug levels compared to purely clinician evaluation or a patient self-report (Becker & Starrels, 2020). A disadvantage of urine testing is "a high risk of adulteration of the sample by the patient to avoid detection of non-compliance with the therapeutic regimen (AACC,

2017)." The table below, adapted from Hoffman (2022) summarizes urine drug testing assays for several drugs.

Drug	Time frame for testing	Substance detected	Potential False-Positives (Varies by Assay)
Amphetamine	1-2 days (acute exposure) 2-4 days (chronic exposure)	Amphetamine	Poor specificity due to structural similarities to many drugs, herbal supplements, and medications, including many nasal decongestants.
Benzodiazepines (Note: No single assay is known to detect all benzodiazepines.)	1-5 days for most benzodiazepines 2-30 days for diazepam	Oxazepam (most common) Various metabolites	Oxaprozin
Cocaine	2 days (acute exposure) 7 days (chronic exposure)	Benzoylecgonine	Coca tea, coca leaves
GHB	< 24 hours	GHB	"Endogenous neurotransmitter naturally present in minute quantities"
Ketamine	1-3 days	Ketamine, norketamine	
LSD	1-3 days	2-oxo-3-hydroxy-LSD	
Marijuana (Note: Synthetic cannabinoids are not usually detected by routine urine assays.)	1-3 days (acute exposure) >1 month (chronic exposure)	11-nor-9-carboxy-Δ^9-THC	Hemp-containing foods or hemp products (e.g. hemp soap) in rare cases
Opioids (Synthetic opioids are not detected by routine opioid screening, though specific assays such as buprenorphine are available.)	1-3 days	Morphine and all natural opioids (e.g. codeine)	Poppy seeds (Note: The threshold for urine detection has been substantially raised to decrease the likelihood of poppy seed false-positives.)
Methadone	1-5 days	Methadone EDDP	Doxylamine
PCP	4-7 days	PCP	Dextromethorphan, diphenhydramine, doxylamine, ketamine, tramadol, venlafaxine

Presumptive urine drug testing (UDT) typically uses an immunoassay where antibodies detect the drug or drug metabolite. This testing can be either qualitative, showing only a positive or negative finding, or semi-quantitative. Immunoassays offer fast turnaround times but can also give false-positive or false-negative results. Federal Workplace Drug Testing Programs usually use higher cutoff values to avoid false-positive results but can increase the likelihood of false-negatives (AACC, 2017). One study reports a false-negative rate of 28% for detecting benzodiazepines (Manchikanti et al., 2010). Another approach is

to utilize orthogonal testing where an initial immunoassay is followed by a spectroscopic assay. This can be used for monitoring compliance in pain management therapy (AACC, 2017). Regardless, proper interpretation of results is imperative. Inadequate physician knowledge of interpretation can limit the efficient use of UDT (Pesce, West, Egan City, & Strickland, 2012); in fact, a single study found that 25 of 88 (28%) of UDT results were susceptible to provider interpretation error when compared to the laboratory toxicologist's interpretation (Chua et al., 2020) (Levy et al., 2007).

Presumptive point-of-care (POC) testing is also available. POC tests use either a urine or saliva sample to screen for drugs in an immunoassay. Like laboratory-based immunoassays, POC testing has lower sensitivity and specificity than definitive drug tests; however, they can provide immediate results to the physician where a negative result typically rules out DOA and a positive result requires confirmatory testing (AACC, 2017). False-positive and false-negative results are even more problematic in POC testing than laboratory-based immunoassays. The clinician must be cognizant of medications—both prescribed and over-the-counter—that can trigger false-positives; for example, over-the-counter nasal inhalers can contain active ingredients that give a potential false-positive for methamphetamine. Moreover, POC testing may not be capable of detecting medications that are metabolites of parent medications (Pesce et al., 2012).

Definitive drug testing typically uses chromatographic and spectroscopic methodologies, including gas chromatography (GC) or liquid chromatography (LC) and tandem mass spectrometry (MS). According to the American Association for Clinical Chemistry (AACC), MS-based assays are traditionally considered the gold standard even though they are both more labor- and time-intensive. Whereas immunoassay-based assays usually only detect a class of compounds, MS-based assays can detect specific drugs in urine samples (AACC, 2017).

Opioids

Opioids are the standard of care for moderate to severe pain, and primarily work by stimulating the μ, δ, or κ opioid receptors in the central nervous system and throughout the body (Owusu Obeng, Hamadeh, & Smith, 2017). The stimulation of these receptors typically causes blocking of pain neurotransmitters such as glutamate and blocks the release of GABA, thereby producing extra dopamine. This extra dopamine also creates a pleasurable effect and possible euphoria (Trescot et al., 2008).

However, due to their mechanism of action, opioids and other pain relievers can cause addiction and are widely abused. According to the CDC, over 142 million prescriptions for opioids were written in 2020 (CDC, 2022b). Although the overall trend in annual opioid prescribing rates have been falling from the peak in 2012 of 81.3 prescriptions per 100 persons to 43.3 per 100 in the most recently reported year (2022) (CDC, 2022b), opioid abuse is still extremely widespread and considered an "epidemic" in the United States. According to the CDC, in 2019, a 4% increase in the number of age-adjusted rate of drug overdose deaths occurred, and 70.6% of all drug overdose deaths involved the use of opioids (CDC, 2022a). In 2019, a total of 70,630 drug overdose deaths occurred in the United States (CDC, 2022a).

The CDC monitors the number of deaths and nonfatal overdoses of opioids in four categories (CDC, 2017b):

- Natural/semi-synthetic opioids, such as morphine and oxycodone, respectively
- Methadone
- Synthetic opioids other than methadone
- Heroin

Immunoassay-based screening tests for opioids typically detect morphine, a common metabolite in natural opioids and heroin; however, synthetic opioids, such as fentanyl, methadone, and tramadol, and semisynthetic opioids, including hydrocodone and oxycodone, are not detected using routine opioid screening. These drugs are detected using a specific screening assay. Previously, poppy seed consumption triggered false-positive results, so the U.S. Substance Abuse Mental Health Services Administration (SAMHSA) raised the urine threshold for morphine from 300 ng/mL to 2000 ng/mL. Additionally, heroin can be distinguished from poppy seed exposure by testing for 6-monoacetylmorphine (6MAM) (Hoffman, 2022). 6-MAM has a short half-life before it metabolizes to morphine; the absence of 6-MAM does not rule heroin use (Pesce et al., 2018)

Non-Opioid Medications Used in Chronic Pain Management

Other non-opioid medications can be used in chronic pain management, including antidepressants, anticonvulsants, neuroleptics, antispasmodics, and muscle relaxants. Tricyclic antidepressants (TCAs), such as nortriptyline, are used in pain management even though the analgesic mechanism is unknown. At times, TCAs may be used as adjuncts to opioid therapy to potentiate the analgesic effect of the opioid for individuals suffering from severe pain and/or diabetic neuropathy. Certain newer anticonvulsants, such as pregabalin and gabapentin, can be used as first-line agents in chronic pain treatment due to favorable side effect profiles. Neuroleptics can be used, especially for patients with psychotic symptomology, but these drugs can have undesirable long-term side effects, including akathisia and tardive dyskinesia. Pain due to muscle spasms in certain individuals may be relieved using muscle relaxants and antispasmodics, including baclofen. These non-opioid medications may be monitored for compliance similarly to their opioid counterparts in patients. The table below lists examples of common non-opioid medications that may be used for pain management (AACC, 2017).

Antidepressants	Anticonvulsants	Neuroleptics	Antispasmodics & Muscle Relaxants
Doxepin Amitriptyline Imipramine Nortriptyline Desipramine Venlafaxine Duloxetine	Phenytoin Gabapentin Pregabalin Carbamazepine Oxcarbazepine Clonazepam	Fluphenazine Haloperidol Chlorpromazine Perphenazine	Baclofen Cyclobenzaprine Carisoprodol

Benzodiazepines and Barbiturates

Due to their anxiolytic and hypnotic properties, tranquilizers, such as benzodiazepines—including Xanax, Valium, and Restoril—have an especially high rate of abuse as they are frequently prescribed for common disorders, such as anxiety and insomnia. Benzodiazepine intoxication has similar features to alcohol intoxication; severe overdose leads to respiratory depression and eventual anoxic brain damage

or death (Weaver, 2015). Benzodiazepines consist of approximately 90% of tranquilizer abuse (Becker & Starrels, 2022) and consisted of approximately 30% of deaths from a pharmaceutical agent in 2010 (Jones et al., 2013). Benzodiazepines are not typically included in the standard urine screening for DOA, but the most common test for benzodiazepines identifies metabolites of 1,4-benzodiazepines like oxazepam. Benzodiazepines that do not metabolize in this manner (such as Xanax) may not be detected. Furthermore, a positive test only indicates a recent exposure to the drug indicated (Greller & Gupta, 2020). The HIV treatment efavirenz gives a false-positive result in benzodiazepine screening; in fact, one study reported that 98% of urine samples of individuals on efavirenz gave a false-positive as compared to only 2% of the control group (Blank et al., 2009). Testing for benzodiazepines is particularly important if opioids or alcohol are involved; 28% of all prescription opioid overdoses in 2015 involved benzodiazepines (Kandel, Hu, Griesler, & Wall, 2017). And, false negative results are often seen in a pain management population in patients prescribed lorazepam and clonazepam because benzodiazepine immunoassays are inadequately sensitive (P. Jannetto et al., 2017).

Although barbiturates, another class of sedatives, are not prescribed as much as in the past, they are still an abusable drug and have use as an anesthetic and anticonvulsant. Barbiturates are also frequently prescribed for headaches, which can lead to physical withdrawal in the form of recurrent headaches (Weaver, 2015). Similar to benzodiazepines, barbiturates can produce a hypnotic and relaxing effect, but euphoria may be a side effect depending on dose (Eskridge & Guthrie, 1997). Its harmful side effects are similar to those of benzodiazepine poisoning (e.g. respiratory depression, slowed mental state) (Greller & Gupta, 2020). The barbiturate immunoassay typically detects secobarbital; the most frequently prescribed barbiturates of phenobarbital, primidone, and butalbital are detected well by barbiturate immunoassays (Algren & Christian, 2015). POC tests, such as the Instant-View® Barbiturate Urine Test, can be used for initial screening but should have confirmatory testing for positive results. According to its package insert, besides phenobarbital, "this test is designed to detect unchanged secobarbital in the urine; however, as with some other analytical methods such as EMIT and RIA, this assay can also detect other commonly encountered barbiturates, depending on the concentration of drug present in the sample. With standard single dose of secobarbital, pentobarbital, or amobarbital, positive results may be identified from 30 hours to 76 hours (ALFA, 2019)." A positive response rate of detection is reported with minimal concentrations of 200 – 300 ng/mL, depending on the barbiturate. The Wondfo Barbiturates Urine Test is another FDA approved POC test which provides results in five minutes. This test can identify 16 drugs including barbiturates and benzodiazepines with a single testing strip (Wondfo, 2020).

Amphetamines

Stimulants, including amphetamines and drugs prescribed for attention-deficit/hyperactivity disorder (ADHD), can be abused due to their euphoric side effects (Moeller et al., 2017). Although there are many different kinds of stimulants, their primary mechanism of action is blocking the dopamine receptor or stimulating release of dopamine (Kampman, 2022). Amphetamine side effects include tachycardia, high blood pressure, and agitation; severe overdose may lead to seizures, hallucinations, or paranoia (Becker & Starrels, 2022). UDTs for amphetamines, such as the DRI® Amphetamines Assay, are immunoassays that detect amphetamine and/or methamphetamine. The DRI® Amphetamines Assay has cutoff levels of 500 ng/mL for amphetamine and 1000 ng/mL for methamphetamine with 58.0% concordance between

the immunoassay and GC/MS at the 500 ng/mL cutoff. The manufacturer states, "a positive result by this assay should be confirmed by another nonimmunological method such as GC, TLC or GC/MS (Microgenics, 2016)." Many false-positives can occur due to the high number of cross-reactants, including over-the-counter medicines and dietary supplements (Hoffman, 2022; Moeller et al., 2017). Even metformin, a medication prescribed to treat diabetes, can give false-positives although the mechanism of cross-reactivity is unknown (Fucci, 2012).

Phencyclidine

Phencyclidine (PCP), a *N*-methyl-*D*-aspartic acid (NMDA) receptor antagonist, is a dissociative anesthetic that can be abused for its euphoric properties. Also known as angel dust, PCP was the first non-natural man-made DOA (Bertron, Seto, & Lindsley, 2018). Throughout the 1980s and 1990s, the use of PCP declined considerably; however, the Drug Abuse Warning Network has reported a 400% increase in emergency room visits due to PCP use in 2005 – 2011 (Moeller et al., 2017). PCP is typically screened using an immunoassay, and qualitative screening tests, such as CEDIA®, report a 100% reactivity at a PCP concentration of 25 ng/mL (Microgenics, 2015). Unfortunately, many compounds can interfere with the PCP immunoassay, including tramadol (Ly, Thornton, Buono, Stone, & Wu, 2012), dextromethorphan, alprazolam, clonazepam, and carvedilol (Rengarajan & Mullins, 2013). Some have reported that diphenhydramine (Benadryl®) also yields false-positive results (Brahm, Yeager, Fox, Farmer, & Palmer, 2010; Levine & Smith, 1990), but other studies have reported it to be statistically insignificant (Rengarajan & Mullins, 2013). The FDA approved Wondfo Phencyclidine Urine Test is an immunochromatographic assay which can identify PCP in human urine with a cutoff of 25 ng/mL (FDA, 2019). Nonetheless, this is considered a preliminary testing method and results should be confirmed with gas chromatography/mass spectrometry (GC/MS) techniques.

Marijuana/THC/Cannabinoids

According to the CDC, the most recent National Survey on Drug Use and Health (NSDUH), conducted by SAMHSA in 2013, showed that approximately 7.5% of people 12 years and older in the U.S. were current users of marijuana, which was up from 5.8% from 2007 (CDC, 2015). Moreover, the CDC reports that the Monitoring the Future (MTF) survey of 8th, 10th, and 12th graders in the U.S. shows that the rate of marijuana usage has remained steady for more than two decades even though many states and municipalities have changed their legislation. Approximately 5.8% of 12th graders reported daily use of marijuana (CDC, 2018a).

Immunoassays for marijuana do not detect tetrahydrocannabinol (THC) directly because THC rapidly metabolizes in vivo (within hours of use). Instead, these assays detect delta-9-THC, a metabolite, which can remain in either the serum or urine for days to weeks, depending on the extent of exposure (Hoffman, 2022). Older urine immunoassays for marijuana were prone to false-positive results (Altunkaya & Smith, 1990; Rollins et al., 1990), but current testing methods are less prone to false-positives (Hoffman, 2022). Due to the legalization of marijuana in certain locales as well as an increase in the potency of the THC in some strains of marijuana, fear of false-positive results due to second-hand smoke has increased. Recent studies show, though, that this is unlikely. None of the individuals tested positive using an immunoassay with a cutoff level of more than 20 ng/mL provided that the room was well-ventilated. If the room was not ventilated, then 4 of 6 individuals tested positive after 1 hour of

exposure if the immunoassay had a cutoff level of 20 ng/mL but only 1 individual tested positive at the federal cutoff level of 50 ng/mL under the same conditions (Cone et al., 2015; Herrmann et al., 2015; Moeller et al., 2017). False positive results for THC have also been caused by medications such as Pantoprazole (Vohra, Marraffa, Wojcik, & Eggleston, 2019). However, Vohra et al. (2019) completed a small study (n=12) and found that oral proton pump inhibitors (such as Pantoprazole) did not cause false-positive THC results with the THC One Step Marijuana Test Strip. This test strip is a rapid chromatographic immunoassay which detects the delta-9-THC metabolite at a cutoff concentration of 50 ng/mL (Xlar, 2002).

Cocaine

Cocaine is an alkaloid produced biosynthetically by *Erthroxylum coca*, which is a plant native to western South America; for thousands of years, South Americans have chewed on the dried coca leaves or consumed coca tea to release cocaine in saliva (Drake & Scott, 2018). Pure cocaine was first isolated in the 1880s and was legal in the United States during the second half of the 19th century (Nelson & Odujebe, 2019). It was once a main ingredient of Coca-Cola. Cocaine is now illegal in the United States; importing coca leaves or coca tea is also illegal in the United States but is legal in other countries. Medicinal use of cocaine is typically limited to use in minor otolaryngologic procedures or as a topical anesthetic (Hoffman, 2021). It has vasoconstrictive properties, making it useful in limiting bleeding during nose and throat surgeries (Nelson & Odujebe, 2022).

Cocaine is a powerful nervous system stimulant and is highly addictive. According to the CDC (2019b), cocaine was involved in almost 1 in every 5 overdose-related deaths in the United States in 2017, leading to 14,000 cocaine-related deaths. In 2016, almost 5 million Americans reported regular cocaine use, which was approximately 2% of the population (CDC, 2019b).

Cocaine has three main metabolites--benzoylecgonine (> 50 %), ecgonine methyl ester (32-49%) and norcocaine (5%) (Nelson & Odujebe, 2019). With benzoylecgonine identified as the major urinary metabolite of cocaine, it is usually tested for in blood, urine, hair, saliva, and meconium. Immunoassays are the most specific technique to detect the cocaine metabolite benzoylecgonine; false-positive results are very uncommon (Hoffman, 2021). Cocaine is metabolized very rapidly and may only be detectable in blood and urine for a few hours; however, benzoylecgonine can be detected in the urine for several days if cocaine use is intermittent or very heavy (Nelson & Odujebe, 2019). Appropriate urine tests distinguish between cocaine use and coca leave/tea use because different metabolites are formed from each. The DRI Cocaine Metabolite Assay, developed by Thermo Fisher, is an FDA-approved enzyme immunoassay that uses a specific antibody to detect benzoylecgonine in urine (FDA, 2018). This immunoassay has a concentration cutoff of 150 ng/mL-300 ng/mL.

Clinical Utility and Validity

For acute clinical management of most patients, DOA monitoring is of limited value. Studies have indicated that in specific settings DOA screening does have value, particularly for drug treatment programs, pain management, and/or psychiatric treatment. A large retrospective study (n = 470 patients) by Michna et al. (2007) showed that 20% of individuals in pain management programs tested positive for illicit substances when random screenings were performed. Further, Knezevic, Khan,

Beiranvand, and Candido (2017) performed a study showing the effect of urine drug testing on patient compliance. Five hundred patients provided supervised urine toxicology samples, 386 of which were compliant with prescribed medications. The patients were educated about their results, and 77 of the noncompliant patients were tested again. Of these 77 patients, 49 had improved compliance (Knezevic et al., 2017). This supports the previous findings of a smaller study by Jamison and colleagues that reported a significant increase in compliance for high-risk chronic pain patients on opioid therapy when monitored by UDT (Jamison et al., 2010). Another study also supports UDT for patients on long-term opioid therapy by showing that "monitoring both urine toxicology and aberrant behavior in chronic-pain patients treated with opioids identified more problem patients than by monitoring either alone (Katz et al., 2003)."

These findings are considerably more favorable than those of the systematic review conducted by Starrels et al. (2010) of eleven different studies that found substantial variation in reduction of opioid misuse in patients with chronic pain. These researchers discovered that "the proportion of patients with opioid misuse after treatment agreements, urine drug testing, or both varied widely (3% to 43%)" and concluded that "relatively weak evidence supports the effectiveness of opioid treatment agreements and urine drug testing in reducing opioid misuse by patients with chronic pain (Starrels et al., 2010)." Even with the controversy, Christo et al. (2011) recommends using an algorithmic approach for urine drug testing where UDT is used to establish "a baseline measure of risk, as well as monitoring for compliance" (Christo et al., 2011), an approach also supported by the Texas Pain Society (Owen, Burton, Schade, & Passik, 2012).

Additionally, other scenarios may utilize DOA testing to alter medical management. Patients with seizure disorders, such as epilepsy, who are on antiepileptic medications that block sodium channels (including phenytoin, lamotrigine, and carbamazepine) could benefit from DOA testing since cocaine can interact pharmacokinetically with these drugs (Smith & McBride, 1999; Wilfong, 2021). DOA screening to check for cocaine can be used prior to administration of beta-adrenergic antagonists. For patients who exhibit acute psychosis with no apparent or known cause, DOA screening can be used to detect possible stimulants (Hoffman, 2021; McClellan & Stock, 2013). Alternatively, psychiatric pre-administration acetaminophen or salicylate screening is deemed unnecessary by Farkas et al. (2021) following their multicenter retrospective study. The authors analyzed 33,439 tests over 10 years from three different Veteran's Administration emergency departments. There were no toxicity diagnoses. The authors suggest that the testing is "unnecessary and wasteful" (Farkas et al., 2021).

For monitoring a drug therapy regimen, some have proposed using quantitative, definitive testing (Couto, Webster, Romney, Leider, & Linden, 2009, 2011; Kell, 1994; Pesce et al., 2012). Small studies by Couto and colleagues reported concordance correlation coefficients of 0.677 (n = 20) for assessing adherence to a hydrocodone regimen and 0.689 (n = 36) for an oxycontin regimen using normalized algorithms (Couto et al., 2009, 2011). Other studies have shown that due to the variability in pharmacokinetics, pharmacodynamics, and pharmacogenetics between individuals, such quantitative testing does not correlate to "patient compliance with a drug dosage using commercial algorithms" (Nafziger & Bertino, 2009). Another study by McEvoy et al. (2014) aiming to assess urine levels of aripiprazole and its metabolites for patients on an aripiprazole regimen, at best, only found an R^2 value

of 0.7 even when adjusted for age, weight, sex, urine creatinine values, height, urine specific gravity, and dosage range. "Unadjusted urine levels of aripiprazole and metabolites are not strongly related to aripiprazole dosing…variance in urine metabolite levels accounted for by medication dose was relatively low for each individual drug/metabolite, [R^2] only 0.13 to 0.23 (McEvoy et al., 2014)." Even the study by Couto notes the limitations concerning pharmacogenetics, excluding any patient who was "determined to be poor, rapid, or ultra-rapid CYP2D6 metabolizers (Couto et al., 2011)."

A study performed by Snyder et al. (2017) assessed the accuracy of enzyme immunoassays (EIAs) for patients being treated for chronic pain. A total of 530 patient samples were taken, and the immunoassays were evaluated for accuracy. The EIAs showed an overall sensitivity of 78.5% (detecting 543 of the 692 LC-MS/MS positives). Unfortunately, "21% of EIA for opiates show false negative results." The authors conclude, "LC-MS/MS methods are superior in terms of sensitivity and number of compounds that can be screened, making this a better method for use in pain management (Snyder et al., 2017)."

A retrospective chart review was conducted by Vopat et al. (2020) for a community-based practice, where 166 patients were examined. Motivated by studies that showed increases in post-operative orthopedic complications associated with pre-operative opioid use, the authors set out to determine whether urine drug screening (UDS) could be an effective screening tool for detecting opioid and illicit drugs prior to joint arthroplasty procedures. In the review, positive UDS results were compared to self-reported history of prescribed opioids. The authors demonstrated using four drug panels that of the 166 patients screened with UDS, 64 (38.6%) tested positive for opiate/opioids, while 7 (4.2%) tested positive for amphetamines, 6 (3.6%) for cannabinoids, and 2 (1.2%) for other drugs, with one participant testing positive across multiple panels. However, it was also admitted that the study may have limited power, given that the population came from a single clinic with a limited number of cases. The narrow detection time of using urine detection screening also presents an issue; for example, drugs such as oxycodone may not be detected if administered more than three days before testing, leading to underestimation. Moreover, the data was not normalized for duration and dosage of opioid use, which are believed to contribute to clinical outcomes. However, the authors ultimately concluded that "With a significant number of patients testing positive for opioids without evidence of a previous prescription, UDS may be beneficial for initial risk assessment for patients undergoing JA procedures" (Vopat et al., 2020).

Palamar, Le, Guarino, and Mateu-Gelabert (2019) completed research to determine the effectiveness of hair versus urine testing to detect or validate drug use. Data from 532 adults was used in this study. All participants reported using heroin or a nonmedical prescription opioid in the past month. Urine samples were obtained from all participants and almost 80% of participants provided hair samples. "Compared to hair testing, urine testing was able to confirm higher proportions of self-reported use of heroin/opioids (85.5% vs. 80.9%), marijuana (73.9% vs. 22.9%), benzodiazepines (51.3% vs. 15.1%), and methadone (77.0% vs. 48.7%), while hair testing was more likely to detect reported cocaine use (66.3% vs. 48.0%) (Ps<.01). Compared to hair testing, urine testing was more likely to detect unreported use of marijuana (11.3% vs. 0.9%), and benzodiazepines (14.4% vs. 5.4%), and hair testing was more likely to detect unreported use of cocaine (27.0% vs. 5.8%) and oxycodone (19.7% vs. 1.4%) (Palamar et al., 2019)." When used together, hair testing increased the detection of cocaine and/or oxycodone use from

14% to 22%. This is not surprising as cocaine is metabolized very quickly and may be undetectable in urine within hours to a few days depending on use (Nelson & Odujebe, 2022).

Böttcher et al. (2019) evaluated the analytical findings in oral fluid after oral fluid heroin intake. The study used 6-acetylmorphine (6-AM) as the target analyte. 2814 samples from 1875 patients were included. At a cut-off of 1 ng/mL "neat" (undiluted) oral fluid, 406 samples contained at least one opiate in the drug screening. 314 of these samples had a measured 6-AM concentration of ≥1 ng/mL. The authors also noted that the positive rates for opiates in oral fluid and urine were identical at 13.5% (in similar populations of patients). The authors concluded that 6-AM "…makes OF drug testing for detecting heroin use more effective than urine drug testing when using highly sensitive mass spectrometry methods (Böttcher et al., 2019)."

A recent study by Krasowski et al. (2020) used data from a College of American Pathologists survey on urine drug testing and screening proficiency to greater understand the strengths and weaknesses of immunoassays in drug testing. The authors note that there is a strong clinical interest for urine drug testing, and that both opiate and amphetamine immunoassays were highly variable regarding cross-reactivity for drugs other than the actual assay calibrator. The authors also found that "urine drug testing availability does not parallel prevailing patterns of drug prescribing and abuse patterns. In particular, specific immunoassays for synthetic opioids and a lower positive cutoff for opiate immunoassays may be underused, whereas immunoassays for barbiturates, methadone, propoxyphene, and phencyclidine may be overused (Krasowski et al., 2020)."

Argoff et al. (2018) published consensus report regarding "urine drug monitoring (UDM) in patients with chronic pain who are prescribed opioids." It is important to note that this publication was sponsored by major toxicology laboratories. The specialists convened were "an interdisciplinary group of clinicians with expertise in pain, substance use disorders, and primary care". They have issued recommendations based on their review of relevant literature, existing guidelines and their clinical experiences in UDM. Their relevant recommendations are listed below:

- "Use definitive UDM testing (e.g., with GC-MS, LC-MS, or LC-MS/MS) as the most accurate method for assessing baseline opioid use and opioid misuse in almost all patients with chronic pain being considered for opioids as well as for ongoing monitoring of patients receiving opioids for chronic pain, unless presumptive testing is required by institutional or payer policies." The guideline acknowledges that "The recommendations in this consensus are intended to be considered together with practical clinical and payer concerns. When required by payers and institutions, immunoassays may be sufficient for monitoring low-risk patients, particularly when clinicians and patients engage in open communication."
- "Perform UDM at baseline in patients prescribed opioids for chronic pain. During ongoing monitoring, perform UDM at least annually for low-risk patients, two or more times per year for moderate-risk patients, and three or more times per year for high-risk patients. Additional monitoring can be performed at any risk level as frequently as necessary according to clinical judgment (Argoff et al., 2018)".

IV. Guidelines and Recommendations

Several organizations recognize the benefit of drug screening/testing for the identification and management of drug misuse and abuse; however, standard guidelines for who should be tested, what test should be used, and how frequently testing should occur, are lacking.

Centers for Disease Control and Prevention (CDC)

In 2016, the CDC published guidelines for prescribing opioids for chronic pain (Dowell et al., 2016). Within the guidelines, the CDC recommends that clinicians should consider urine drug testing prior to discontinuing opioids to determine possibility of withdrawal. The CDC also recommends that "when prescribing opioids for chronic pain, clinicians should use urine drug testing before starting opioid therapy and consider urine drug testing at least annually to assess for prescribed medications as well as other controlled prescription drugs and illicit drugs (recommendation category: B, evidence type: 4)." The CDC states that "urine drug tests can provide information about drug use that is not reported by the patient. In addition, urine drug tests can assist clinicians in identifying when patients are not taking opioids prescribed for them, which might in some cases indicate diversion or other clinically important issues such as difficulties with adverse effects (Dowell et al., 2016)."

Concerning the frequency of urine drug testing, they state, "While experts agreed that clinicians should use urine drug testing before initiating opioid therapy for chronic pain, they disagreed on how frequently urine drug testing should be conducted during long-term opioid therapy. Most experts agreed that urine drug testing at least annually for all patients was reasonable. Some experts noted that this interval might be too long in some cases and too short in others, and that the follow-up interval should be left to the discretion of the clinician. Previous guidelines have recommended more frequent urine drug testing in patients thought to be at higher risk for substance use disorder. However, experts thought that predicting risk prior to urine drug testing is challenging and that currently available tools do not allow clinicians to reliably identify patients who are at low risk for substance use disorder (Dowell et al., 2016)."

- The CDC also published a guideline "Quality Improvement and Care Coordination: Implementing the CDC Guideline for Prescribing Opioids for Chronic Pain" to provide guidance to healthcare systems and practice leaders. In it, the CDC details specific procedures to take when "unexpected results" appear.
- When the UDT is negative for a prescribed opioid, the CDC recommends repeating the test "using chromatography" and to specify the drug of interest.
- When the UDT is positive for a non-prescribed opioid, benzodiazepines, or illegal drugs, the CDC recommends repeating the UDT regularly.
- When the urine sample has a creatinine level of <2-3 mmol/L or < 20 mg/dL, the CDC recommends repeating the UDT.
- When the urine sample is cold, the CDC recommends repeating the UDT (CDC, 2018b).

American Academy of Family Physicians (AAFP)

The AAFP published in 2019 recommendations concerning ordering and interpreting urine drug tests. They state, "Several federal and state regulations have been enacted that recommend or require urine drug testing in patients receiving long-term opioid therapy. Similar guidance may apply to patients receiving long-term benzodiazepine or stimulant therapy. (Kale, 2019)." They state that the frequency of urine drug testing depends on individual risk factors and is ultimately left to the attending physician; however, they do state a recommended frequency for urine drug testing given in the table below:

Recommended Frequency for Urine Drug Testing (Kale, 2019)	
Level of misuse risk	Frequency of testing
Low (no risk factors)	Every 6 to 12 months
Moderate	Every 3 to 6 months
High (mental health disorder, substance use disorder, prior opioid misuse, aberrant behavior*) or opioid dosage >120 morphine milligram equivalents	Every 1 to 3 months
*Aberrant behavior includes, but is not limited to, lost prescriptions, multiple requests for early refills, opioid prescriptions from multiple physicians, unauthorized dose escalation, and apparent intoxication.	

They state the following clinical recommendation: "Urine drug testing can be used to monitor compliance with prescribed therapy and detect the use of nonprescribed and illicit substances, especially opioids, benzodiazepines, and heroin."

In 2020, the AAFP provided a clinical preventive service recommendation on screening for opioid use disorder, stating that "The AAFP recommends that clinicians selectively screen and refer adults age 18 years and older to OUD treatment after weighing the benefits and harms of screening and treatment. Clinicians should consider all benefits and harms including health, social, and legal outcomes. Screening programs should only be implemented if services for accurate diagnosis, effective treatment, and psychosocial supports can be offered or referred" (AAFP, 2020). This recommendation falls under the category of grade C, or the recommendation provides "at least moderate certainty that the net benefit is small".

Federation of State Medical Boards (FSMB)

The FSMB indicates in their Guidelines for Chronic Use of Opioid Analgesics policy that for patients being prescribed opioids for chronic pain management that the initial workup should include a system review and relevant physical examination, as well as laboratory investigations as indicated (FSMB, 2017). They also note the utility of periodic and unannounced testing for monitoring adherence to the patient's treatment plan and to detect non-prescribed drugs. Regarding frequency of testing, "Patients being treated for addiction should be tested as frequently as necessary to ensure therapeutic adherence, but for patients being treated for pain, clinical judgment trumps recommendations for frequency of testing (FSMB, 2017)."

Additionally, relative to how testing should be performed, the Federation of State Medical Boards notes that POC tests have significant limitations in both sensitivity and specificity, and therefore "the use of point of care testing for the making of more long term and permanent changes in management of people with the disease of addiction and other clinical situations may not be justified until the results of confirmatory testing with more accurate methods … are obtained." They do state, "Urine may be the preferred biologic specimen for testing because of its ease of collection and storage and the cost-effectiveness of such testing. When such testing is conducted as part of pain treatment, forensic standards are generally not necessary and not in place (FSMB, 2017)." They also note that initial testing could be done using immunoassays and followed up by a more specific technique, such as GC/MS or other chromatography-based technique. They highlight the importance of knowing specific drug and metabolites, "not just the class of drug" for the pain management.

American Academy of Pain Medicine (AAPM)

The AAPM notes that "urine and/or blood drug screening… may be helpful in ruling out the issue of diversion," along with other non-testing actions. They also note that "when appropriate, the patient should undergo a baseline drug screening exam." They highlight the importance of random urine drug screening for the ongoing monitoring of patient compliance to the treatment plan (AAPM, 2013).

The AAPM also co-sponsored guidelines with the American Association for Clinical Chemistry in 2018. These guidelines by Langman and Jannetto (2018) are shown below.

American Association for Clinical Chemistry (AACC)

In 2017, the AACC published their guidelines titled *Using Clinical Laboratory Tests to Monitor Drug Therapy in Pain Management Patients* (P. Jannetto et al., 2017). These guidelines were reaffirmed in 2018 and co-sponsored by the AAPM (Langman & Jannetto, 2018). The AACC lists medications in tiers to guide ordering of tests. Tier 1 is "routine monitoring" and includes frequently abused drugs as well as drugs frequently prescribed to pain management patients. Benzodiazepines, amphetamines, and barbiturates are in this tier. Anticonvulsants and antidepressants fall in tier 2, which is as follows: "High-risk patients with known history of abuse for this medication or prevalence of drug use is endemic to local region, risky polypharmacy, multiple providers, or if prescribed and patient shows lack of efficacy or toxicity" (P. Jannetto et al., 2017). Antipsychotics fall in tier 3: which should be ordered "as clinically indicated."

The NACB [AACC] lists their recommendations with a grade for the quality of evidence as well as the strength of recommendation. An A represents a strong recommendation, a B is moderate recommendation, and C is a recommendation against. For the quality of evidence, an "I" represents "consistent results from well-designed, well-conducted studies in representative populations" whereas an "II" means "Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence." The NACB's recommendations are as follows (P. Jannetto et al., 2017; Langman & Jannetto, 2018):

- "Testing biological specimens for drugs/drug metabolites is recommended and effective for detecting the use of relevant over-the-counter, prescribed and non-prescribed drugs, and illicit

substances in pain management patients. Laboratory testing does not specifically identify most other outcomes, but should be used in conjunction with additional information to detect other outcomes in pain management patients. Strength of Recommendation: A; Quality of Evidence: I"

- "More frequent laboratory testing is recommended for patients with a personal or family history of substance abuse, mental illness, evidence of aberrant behavior, or other high-risk characteristics. Strength of Recommendation: A; Quality of Evidence: II"
- "Laboratory testing is recommended to identify the use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. However, it does not effectively identify all non-compliance with the prescribed regimen. No single monitoring approach provides adequate information about the pattern or dose of patient drug use. Safest prescribing habits should include a combination of tools and laboratory test results to correctly detect outcomes. Strength of recommendation: A; Quality of evidence: III (pain management population), II (substance abuse disorder monitoring population)"
- "Laboratory testing is more effective than other physician tools for the detection of relevant over-the-counter, prescribed and non-prescribed drugs, and illicit substances in pain management patients and should be used routinely to monitor compliance. Strength of recommendation: A; Quality of evidence: II"
- "Urine testing is recommended for the detection of relevant over-the-counter medications, prescribed and nonprescribed drugs, and illicit substances in pain management patients. Strength of recommendation: B; Quality of evidence: II"
- "Based on level II evidence, baseline drug testing should be performed prior to initiation of acute or chronic controlled substance therapy. In addition, random drug testing should be performed at a minimum of one to two times a year for low-risk patients (based on history of past substance abuse/addiction, aberrant behaviors, and opioid risk screening criteria), with increasing frequency for higher-risk patients prescribed controlled substances. Strength of Recommendation: A; Quality of Evidence: II"
- "Serum or plasma is an acceptable alternate matrix for the detection of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients with end-stage renal failure (anuria). For dialysis patients, the blood (serum/plasma) should be collected prior to dialysis. Oral fluid testing can also be used for selected drugs (e.g. amphetamine, benzodiazepines, buprenorphine, tetrahydrocannabinol, cocaine, codeine, hydrocodone, hydromorphone, methadone, morphine, oxycodone, and oxymorphone). Strength of recommendation: A; Quality of evidence: III"
- "While definitive testing is recommended and preferred, urine immunoassays performed on laboratory-based analyzers offer some clinical utility to detect the use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. However, physicians using immunoassay-based tests (especially amphetamine, benzodiazepine, and opiate immunoassays) must reference the package insert if testing in the physician's office or consult with laboratory personnel to evaluate the assay's capabilities and limitations for detecting specific medications within a drug class to prevent incorrect interpretation and to determine when additional testing is necessary. Strength of Recommendation: B; Quality of Evidence: II"

- "Qualitative definitive tests should be used over immunoassays since they are more effective at identifying relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Strength of Recommendation: A; Quality of Evidence: II"
- "Qualitative definitive tests should be used when possible over immunoassays for monitoring use (compliance) to relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients due to their superior sensitivity and specificity. Strength of Recommendation: A; Quality of Evidence: II"
- "POC (oral/urine) qualitative presumptive immunoassays offer similar performance characteristics to laboratory-based immunoassays and can detect some over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. However, physicians using POC testing must reference the POC package insert and/or consult laboratory personnel to accurately determine the assay's capabilities (especially amphetamine, benzodiazepine, and opiate immunoassays) and understand the limitations for detecting specific medications within a drug class to prevent incorrect assumptions or interpretation and to determine when additional testing is necessary. Strength of Recommendation: B; Quality of Evidence: II"
- "Qualitative immunoassay drug testing prior to prescribing controlled substances can be used to identify some illicit drug use and decrease adverse outcomes in pain management patients. Strength of Recommendation: B; Quality of Evidence: II"
- "Random urine testing for relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances is recommended to detect outcomes in pain management patients. Strength of Recommendation: A; Quality of Evidence: III (pain management population), II (substance abuse disorder monitoring population)"
- "Appropriately performed and interpreted urine POC immunoassay testing can be cost-effective for detecting use or inappropriate use of some over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Strength of Recommendation: B; Quality of Evidence: II"
- "Firstline definitive testing (qualitative or quantitative) is recommended for detecting the use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Strength of recommendation: A; Quality of evidence: II"
- "Recommend definitive testing for any immunoassay (laboratory-based or POC) result that isn't consistent with the clinical expectations in a pain management patient. Strength of recommendation: A; Quality of evidence: III"
- "Quantitative definitive urine testing is not more useful at detecting outcomes in pain management patients compared to qualitative definitive urine testing. Furthermore, quantitative definitive urine testing should not be used to evaluate dosage of administered drug or adherence to prescribed dosage regimen. However, quantitative urine definitive testing is recommended to identify variant drug metabolism, detect pharmaceutical impurities, or metabolism through minor routes. Quantitative results may also be useful in complex cases to determine the use of multiple opioids, confirm spiked samples, and/or rule out other sources of exposure (e.g. morphine from poppy seeds). Strength of recommendations: A; Quality of evidence: II"

- "The use of lower limit-of-detection cutoff concentrations can be more effective to detect use (either partial or full compliance) or the lack of use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients, especially those taking lower dosages. Strength of Recommendation: B; Quality of Evidence: II (P. Jannetto et al., 2017; Langman & Jannetto, 2018)"

American Pain Society/American Academy of Pain Medicine

The American Pain Society and American Academy of Pain Medicine joint guidelines panel released their opioid treatment guidelines titled *Clinical Guidelines for the Use of Chronic Opioid Therapy in Chronic Non-cancer Pain* in 2009. They addressed the monitoring of controlled substances use via UDT as part of a chronic opioid treatment (COT) program. The authors recommend periodic urine drug screening and suggest that random urine drug screens may be more informative than scheduled or routine testing. The guideline section on monitoring (Section 5) states:

- "5.1: Clinicians should reassess patients on COT periodically and as warranted by changing circumstances. Monitoring should include documentation of pain intensity and level of functioning, assessments of progress toward achieving therapeutic goals, presence of adverse events, and adherence to prescribed therapies (strong recommendation, low-quality evidence).
- 5.2: In patients on COT who are at high risk or who have engaged in aberrant drug-related behaviors, clinicians should periodically obtain urine drug screens or other information to confirm adherence to the COT plan of care (strong recommendation, low-quality evidence).
- 5.3: In patients on COT not at high risk and not known to have engaged in aberrant drug-related behaviors, clinicians should consider periodically obtaining urine drug screens or other information to confirm adherence to the COT plan of care (weak recommendation, low-quality evidence). Clinicians should periodically reassess all patients on COT. Regular monitoring of patients once COT is initiated is critical because therapeutic risks and benefits do not remain static" (Chou et al., 2009).

The American Pain Society guidelines state that for individuals at low-risk for adverse outcomes, quarterly or semi-annual monitoring is sufficient. The risk for abuse may be measured using standard tools, such as the Screener and Opioid Assessment for Patients with Pain (SOAPP) and the Opioid Risk Tool. These types of tools may help clinicians assess the suitability of long-term opioid therapy for chronic pain patients and may help differentiate those patients who require more clinician monitoring while on long-term opioid therapy. Both tools may be self-administered at or prior to an office visit, or completed as part of an interview with a nurse, physician or psychologist (Chou et al., 2009).

American Society of Interventional Pain Physicians (ASIPP)

ASIPP issued evidence-based clinical practice guidelines to improve the quality of care through responsible opioid prescribing in non-cancer pain. They have described evidence assessment followed in Part 1 of the guidelines and the recommended guidance in Part 2.

ASIPP provides 11 recommendations including drug cut-offs and detection limits for drugs of abuse, drug cross-reactants, guidance on interpretation of unexpected results for urine drug testing and urine drug testing algorithm. In their algorithm, ASIPP proposes to perform baseline assessment of the patient with chronic pain using POC immunoassay. Then, depending on the result to continue either compliance

monitoring with random POC immunoassay in 1-3 months if initial results were appropriate or explained, followed-up with random testing in 6-12 months if the result remains appropriate. In the case when inappropriate or unexplained results are obtained, confirmatory testing is proposed with repeat urine drug testing in one month or next appointment (Manchikanti et al., 2012).

In their recommendation 1D, level of evidence good, ASIPP states: "Urine drug testing (UDT) must be implemented from initiation along with subsequent adherence monitoring to decrease prescription drug abuse or illicit drug use when patients are in chronic pain management therapy." Additionally, they state, "In order to reduce prescription drug abuse and doctor shopping, adherence monitoring by UDT and PMDPs provide evidence that is essential to the identification of those patients who are non-compliant or abusing prescription drugs or illicit drugs." Level of evidence is fair (Manchikanti et al., 2012).

Agency Medical Directors' Group (AMDG)

The AMDG published an Interagency Guideline on opioid dosing for chronic non-cancer pain. This guideline and related expert commentary support low-risk individuals having UDT up to once per year, moderate risk up to 2 per year, high risk individuals up to 3-4 tests per year, and individuals exhibiting aberrant behaviors should be tested at the time of the office visit (AMDG, 2015).

Supplemental guidance on prescribing opioids for postoperative pain was published by the AMDG in 2018. Specific opioid testing methods are not mentioned in these guidelines (AMDG, 2018).

Wisconsin Worker's Compensation Patient Care

Wisconsin's Worker's Compensation program recommends for any worker's compensation patient who will need opioid treatment for a period of more than 90 days, that the treating physician should follow these guidelines and or consider referral to a Pain Management specialist. In their document, they state that "urine drug screening before starting chronic opioid therapy is imperative" to verify that patient is not using illegal substances. In addition, according to their guidelines, compliance monitoring is mandatory for all patients on chronic opioid therapy with several tools including urine drug screen for the first visit and with aberrant behavior and unannounced urine drug screens thereafter (DWD, 2013).

American Society of Addiction Medicine (ASAM)

ASAM states quantification (assessing specific concentration of a drug) should not be used to determine adherence with a specific dosage or formulation regimen. There are, however, specific reasons for obtaining quantitative data. For example, quantification can help a clinician decide why the other opioids are present. Serial creatinine-corrected quantitative values can help the clinician distinguish cessation of drug use from continued drug excretion from ongoing drug use. Finally, the guidelines note that state laws may also guide testing decisions (ASAM, 2013).

In 2017, the ASAM recommended drug testing as "an important supplement to self-report because patients may be unaware of the composition of the substance(s) they have used (Jarvis et al., 2017)." They also recommend to not rely on the SAMHSA-5 panel as a routine drug panel. ASAM states that urine testing for amphetamines and benzodiazepines may be helpful when assessing potential use. The

society also emphasizes that the results must be carefully analyzed due to specificity limitations in both immunoassays.

With regards to general testing, ASAM recommends random, unannounced testing as opposed to scheduled ones. They recommend, "presumptive testing should be a routine part of initial and ongoing patient assessment." Concerning definitive drug testing, they recommend, "Definitive testing techniques should be used whenever a provider wants to detect specific substances not identified by presumptive methods, quantify levels of the substance present, and refine the accuracy of the results. Definitive testing should be used when the results inform clinical decisions with major clinical or nonclinical implications for the patient (e.g. treatment transition, changes in medication therapies, changes in legal status) (Jarvis et al., 2017)." ASAM also considers GC-MS and LC-MS testing for confirmation of a presumptive positive test. For patients in substance abuse treatment, ASAM recommends frequent random testing (at least weekly) initially. Once the patient is stable in treatment, then the frequency can decrease (to at least monthly).

New York State Office of Addiction Services and Supports (OASAS)

The OASAS published guidelines on toxicology testing during treatment for substance use disorders. The guidelines specify that toxicology testing may include urine, blood, breath, oral fluid, sweat, and hair, but note that urine testing is the come common and validated method.

The guidelines outline when toxicology testing should be completed. Toxicology testing should be used during initial diagnosis to determine which substances have been used recently and to guide further clinical decisions and testing. Toxicology testing should also be used to determine the level of care necessary, monitor treatment progress, monitor the use of secondary substances, to determine if symptoms are related to a substance, and when documentation of current substances is needed. Lastly, toxicology testing should be used for "return to use prevention" to help the patient make informed decisions.

The guidelines go on to outline how often toxicology tests should be administered. Toxicology testing should be completed upon patient intake, as requested by the patient, at random intervals throughout treatment (decreasing in frequency), and as clinically needed. The guidelines note that the window of detection for each substance must be considered when determining toxicology testing frequency. Participants in the New York State Drug Courts may require toxicology testing twice a week. Patients in opioid treatment programs require "at least eight random toxicology tests per year" (OASAS, 2021).

Texas Pain Society

The Texas Pain Society released detailed guidelines concerning urine drug testing (UDT) and its use in the practice of pain management. They do not recommend a prescribed regimen of UDT but rather leave it to the discretion of the physician. They do recommend random UDT over scheduled UDT. Concerning what should be included in a UDT, "Elements of UDT may include specific gravity, temperature at the time of sample collection, pH, creatinine concentration, and mass spectroscopic confirmatory testing for the following agents: opioids (fentanyl, oxycodone, oxymorphone, tramadol, methadone, hydrocodone, hydromorphone, morphine, codeine, propoxyphene, meperidine, buprenorphine, tapentadol, 6-mono-acetyl morphine [6-MAM])…"(Owen et al., 2012). Concerning the

frequency of conducting UDTs, they recommend 1-2 tests per year for low-risk patients; 3-4 tests per year for moderate-risk patients; and "4 [per year] or every month, office visit, or every drug refill" for high-risk patients.

2014 Annals of Internal Medicine Review

In 2014 Nuckols and colleagues released an extensive review of guidelines on prescribing and monitoring opioids from more than ten different societies and organizations in the *Annals of Internal Medicine*. No consensus concerning urine drug monitoring or testing was noted across all guidelines; in fact, the APS-AAPM noted to use UDT only "if risk is high; consider otherwise." The NOUGG recommends that, if UDT is used, to consider pros and cons (expert consensus). The Colorado Division of Workers Compensation requires mandatory UDT. The VA/DoD and ASIPP uses UDT to establish a baseline followed by random testing during treatment whereas the ACOEM and UMHS uses UDT to establish a baseline followed by either a minimum of quarterly testing or annual testing, respectively (Nuckols et al., 2014).

Substance Abuse and Mental Health Services Administration (SAMHSA)

These guidelines are for the certification for opioid treatment programs (OTPs). OTPs require certification before they can dispense opioids to treat opioid addiction. SAMHSA recommends benzodiazepines and amphetamines at a minimum be tested before admission to any opioid treatment program. Barbiturates are also strongly recommended to be tested at regular intervals during the program. Testing is not limited to these classes of drugs and may vary from individual to individual (SAMHSA, 2007).

SAMHSA federal guidelines for opioid treatment programs were updated in 2015. These guidelines state that "Clinicians should determine the drug-testing regimen by analyzing community drug-use patterns and individual medical indications. It is strongly recommended that benzodiazepines, barbiturates, and alcohol (using the ethyl glucuronide test) be included in drug screening and testing panels (SAMHSA, 2015, 2017)." The guidelines also state that "OTPs often perform onsite point of collection (POC) tests using sensitive and automated immunoassay (IA) technologies that screen urine or oral fluid samples for a relatively narrow range of drug classes (e.g. amphetamines, barbiturates, benzodiazepines, opioids) and a limited number of specific drugs. POC tests such as IAs have a place in clinical decision making, but are not by themselves adequate to satisfy the regulatory requirements for drug use testing services (SAMHSA, 2015)."

In 2020, SAMHSA published guidelines regarding use of oral fluid for federal workplace drug testing programs. In it, they remarked that "The Department believes that collecting and testing oral fluid specimens according to the requirements in these Guidelines is an efficient means to detect illicit drug use and ensures that the oral fluid test results are forensically and scientifically supportable." SAMHSA writes that several reasons demanded the need for regulation of oral fluid testing, such as the need to decrease invalid urine tests. SAMHSA writes that an oral fluid specimen may be used for the following reasons: "a federal agency applicant/preemployment test, a random test, a reasonable suspicion/cause test, a post-accident test, a return to duty test, or a follow-up test. (SAMHSA, 2020)".

Regarding the tests that should be conducted on an oral fluid specimen, a federal agency

> "(a) Must ensure that each specimen is tested for marijuana and cocaine as provided in the drug testing panel described under Section 3.4;
> (b) Is authorized to test each specimen for other Schedule I or II drugs as provided in the drug testing panel;
> (c) Is authorized upon a Medical Review Officer's request to test an oral fluid specimen to determine specimen validity using, for example, a test for a specific adulterant;
> (d) Is authorized to test each specimen for one or more biomarkers as provided in the biomarker testing panel described under Section 3.4; and
> (e) If a specimen exhibits abnormal characteristics (*e.g.,* unusual odor or color, semi-solid characteristics), causes reactions or responses characteristic of an adulterant during initial or confirmatory drug tests (*e.g.,* non-recovery of internal standard, unusual response), or contains an unidentified substance that interferes with the confirmatory analysis, then additional testing may be performed" (SAMHSA, 2022).

Section 3.4 refer to drug and biomarker test analytes and cutoffs for undiluted (neat) oral fluids, and a screenshot is included below.

Initial test analyte	Initial test cutoff [1]	Confirmatory test analyte	Confirmatory test cutoff concentration
Marijuana (THC) [2]	4 ng/mL [3]	THC	2 ng/mL
Cocaine/Benzoylecgonine	15 ng/mL	Cocaine Benzoylecgonine	8 ng/mL 8 ng/mL
Codeine/Morphine	30 ng/mL	Codeine Morphine	15 ng/mL 15 ng/mL
Hydrocodone/Hydromorphone	30 ng/mL	Hydrocodone Hydromorphone	15 ng/mL 15 ng/mL
Oxycodone/Oxymorphone	30 ng/mL	Oxycodone Oxymorphone	15 ng/mL 15 ng/mL
6-Acetylmorphine	4 ng/mL [3]	6-Acetylmorphine	2 ng/mL
Phencyclidine	10 ng/mL	Phencyclidine	10 ng/mL
Amphetamine/Methamphetamine	50 ng/mL	Amphetamine Methamphetamine	25 ng/mL 25 ng/mL
MDMA [4] /MDA [5]	50 ng/mL	MDMA MDA	25 ng/mL 25 ng/mL

[1] For grouped analytes (i.e., two or more analytes that are in the same drug class and have the same initial test cutoff):

Immunoassay: The test must be calibrated with one analyte from the group identified as the target analyte. The cross reactivity of the immunoassay to the other analyte(s) within the group must be 80 percent or greater; if not, separate immunoassays must be used for the analytes within the group.

Alternate technology: Either one analyte or all analytes from the group must be used for calibration, depending on the technology. At least one analyte within the group must have a concentration equal to or greater than the initial test cutoff or, alternatively, the sum of the analytes present (i.e., equal to or greater than the laboratory's validated limit of quantification) must be equal to or greater than the initial test cutoff.

[2] An immunoassay must be calibrated with the target analyte, Δ-9-tetrahydrocannabinol (THC).

[3] Alternate technology (THC and 6-AM): The confirmatory test cutoff must be used for an alternate technology initial test that is specific for the target analyte (i.e., 2 ng/mL for THC, 2 ng/mL for 6-AM).

[4] Methylenedioxymethamphetamine (MDMA).

[5] Methylenedioxyamphetamine (MDA).

American Association for the Treatment of Opioid Dependence Inc. (AATOD)

The AATOD recommends cessation of benzodiazepines before admission to an opioid treatment program (OTP). Gradually tapering off to a lower dose is also acceptable, but benzodiazepine use must be addressed prior to an OTP admission. The AATOD recommends toxicology screening for benzodiazepines, as well as routine checks of each state's Prescription Monitoring Drug Program. Confirmatory testing may also be used (AATOD, 2017).

Department of Health and Human Services (HHS)

The HHS has provided guidelines on Federal Workplace Drug Testing Programs. Federal agencies must comply with these guidelines by October 1, 2017. Each specimen must be tested for marijuana and cocaine metabolites, phencyclidine, opioids, and amphetamines. Validity tests such as creatinine, specific gravity, pH, and oxidizing adulterants must be performed on each specimen. Additional testing must be performed on any abnormal specimens, such as unusual color or smell. Additional drugs may be tested for if the federal agency has reasonable suspicion or the testing is done post-accident. Additional approval is required. The criteria for an adulterated specimen are as follows: pH <4 or >11, nitrite concentration >500 mcg/mL, chromium concentration >50 mcg/mL, and including but not limited to halogens, surfactants, pyridines. Unusual creatinine or specific gravity may also cause a specimen to be invalid (HHS, 2017).

American Academy of Child and Adolescent Psychiatry (AACAP)

AACAP notes, "Toxicology screens are indicated for acute onset or exacerbations of psychosis when exposure to drugs of abuse cannot otherwise be ruled out. Genetic testing is indicated if there are associated dysmorphic or syndromic features (McClellan & Stock, 2013)."

World Federation of Societies of Biological Psychiatry (WFSBP)

The WFSBP states that drug screening (urine and blood) should be sought for schizophrenia patients as "presence of substance abuse or dependence is often not recognized and systematically assessed, especially if such a patient is seen during an acute psychotic episode" (WFSBP, 2015).

National Institute for Health and Care Excellence (NICE)

NICE notes that appropriate blood tests (such as glucose, calcium, and so on) should be considered in adults to identify potential causes or co-morbidities. NICE also acknowledges that children may be tested to exclude other diagnoses or determine an underlying cause of the epilepsy. They also state, "In children and young people, other investigations, including blood and urine biochemistry, should be undertaken at the discretion of the specialist to exclude other diagnoses, and to determine an underlying cause of the epilepsy (NICE, 2018, 2020)."

American Academy of Neurology (AAN)

The AAN states that "toxicology testing may be considered in children with status epilepticus, when no apparent etiology is immediately identified" (AAN, 2018).

Department of Veterans Affairs/Department of Defense (VA/DOD)

In 2015, the VA/DOD issued recommendations surrounding the screening and treatment of substance use disorders. In it, it was recommended that "For patients in general medical and mental healthcare settings, we recommend screening for unhealthy alcohol use annually using the three-item Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) or Single Item Alcohol Screening Questionnaire (SASQ)" (DVA & DOD, 2015).

In 2017, the VA/DOD published clinical practice guidelines for opioid therapy for chronic pain. These guidelines recognize that "UDT and confirmatory testing is an additional method of examining for patient substance misuse" (DOD, 2017). The guidelines also state that "It is critical that the UDT and confirmatory testing be done in a timely, confidential, accurate, and easily available manner to assure the prescribers, patients, and public that safety, fairness, and trust are being addressed (DOD, 2017)." The VA/DOD also recognizes the three main types of UDTs: immunoassay, GC/MS confirmatory testing and LC/MS confirmatory testing.

With respect to antepartum use of alcohol, cigarettes, illicit drugs, and the like, these joint guidelines state "We recommend screening for use of tobacco, alcohol, illicit drugs, and unauthorized use of prescription medication because their use is common and can result in adverse outcomes" (strong recommendation) (DOD, 2018).

Anxiety Disorders Association of Canada (ADAC)

The ADAC recommends urine toxicology as part of the patient's baseline investigations if warranted. This urine toxicology assessment applies to anxiety and other related disorders, which include "panic disorder, agoraphobia, GAD, selective mutism, separation anxiety disorder, SAD (social phobia), specific phobia, substance/medication-induced anxiety disorder, as well as anxiety disorder due to another medical condition or not elsewhere classified" (Katzman et al., 2014).

American Psychiatric Association, Practice Guidelines for the Psychiatric Evaluation of Adults, 3rd Edition (APA, 2016)

The Association acknowledges that urine toxicology may provide clues to substance abuse during an initial psychiatric evaluation (APA, 2016).

World Health Organization (WHO)

The WHO released an intervention guideline for mental, neurological, and substance use disorder in non-specialized health settings. The WHO states that urine testing may be considered to confirm abstinence and to "consider occasional urine testing to confirm non-use." Under the section concerning the investigation of chronic drug use, they state to consider using urine drug screens "for emergency cases, a urine drug screen should be conducted whenever intoxication, withdrawal, or overdose is suspected, especially in cases when the person is unable to convey what they have ingested (WHO, 2016)." The WHO lists the following substances as psychoactive substances: alcohol, benzodiazepines, opioids, tobacco, cocaine, methamphetamines, amphetamine-type stimulants, khat, cannabis, tramadol, "volatile" solvents, MDMA, and hallucinogens.

American College of Obstetricians and Gynecologists (ACOG)

ACOG states that additional research is needed to better understand the effects of universal urine screening on clinical outcomes and recommend validated verbal screening tools instead. ACOG acknowledges that urine drug testing has been used to identify substance abuse and should only be performed in compliance with state's laws and with patient consent. ACOG also lists the following recommendations:

- "Screening for substance use should be part of comprehensive obstetric care and should be done at the first prenatal visit in partnership with pregnant woman. Screening based only on factors, such as poor adherence to prenatal care or prior adverse pregnancy outcome, can lead to missed cases and may add to stereotyping and stigma. Therefore, it is essential that screening be universal."
- "Routine screening should rely on validated screening tools, such as questionnaires, including 4Ps, NIDA, Quick Screen, and CRAFFT (for women 26 years or younger) (ACOG, 2017).

ACOG explicitly states, "Routine urine drug screening is controversial for several reasons. A positive drug test result is not in itself diagnostic of opioid use disorder or its severity. Urine drug testing only assesses for current or recent substance use; therefore, a negative test does not rule out sporadic substance use… Health care providers should be aware of t heir laboratory's test characteristics and request that confirmatory testing with mass spectrometry and liquid or gas chromatography be performed as appropriate (ACOG, 2017)."

Society of Obstetricians and Gynaecologists of Canada (SOGC)

The SOGC recommends periodic drug screening for all pregnant women and all women of childbearing age (III-A). The recommended method of drug screening is a urine toxicology screen (II-2A); however, they state that prior to maternal drug toxicology testing is ordered that informed consent be obtained (III-B) (Wong et al., 2011).

Updated 2017 SOGC guidelines state that "When testing for substance use is clinically indicated, urine drug screening is the preferred method (II-2A) (Ordean et al., 2017)."

Canadian Paediatric Society (CPS)

In 2017, the CPS—in a position statement dealing with cannabis in Canada's children and youth—urged the following recommendation for healthcare providers: "Screen all children and youth for cannabis exposure and/or use and educate adolescents and families on the health risks and harms associated with cannabis" (Grant & Bélanger, 2017).

The CPS within their 2018 guidelines on ADHD in children and youth state, "Children with ADHD may also experience comorbid depressive symptoms, particularly as they approach adolescence and adulthood. There is increasing evidence of heterotypic continuity between these two conditions, suggesting they may represent the same underlying construct for some children. The validity of BD diagnosis, particularly when broadly defined, remains controversial in preadolescent children… There is an increase in SUDs as children with ADHD reach adolescence and adulthood. It is possible that substance use occurs as an attempt to self-medicate. The treatment of ADHD comorbid with a SUD is

complicated by risks for misuse and diversion of prescription stimulants (Gray et al., 2018)." The CPS makes no statement regarding mode of testing or frequency of testing.

Pain Management Best Practices Inter-Agency Task Force Report

The Pain Management Best Practices Inter-Agency Task Force recognizes the importance of screening and monitoring in pain management in identifying and reducing the risk of substance misuse, abuse, and overdose, as well as improving overall patient care. As such, they include a series of gaps in care and related recommendations regarding screening, including the following:

"GAP 1: Comprehensive screening and risk assessment of patients are time-consuming but vital for proper evaluation of their chronic pain conditions. Lack of sufficient compensation for time and payment for services have contributed to barriers in best practices for opioid therapy.

• RECOMMENDATION 1A: Encourage CMS and private payers to provide sufficient compensation for time and payment for services to implement the various screening measures (e.g., extensive history taking, review of medical records, PDMP query, urine toxicology screenings, when clinically indicated). These are vital aspects of risk assessment and stratification for patients on opioids and other medications.
• RECOMMENDATION 1B: Consider referral to pain, mental health, and other specialists, including addiction medicine-trained physicians when high-risk patients are identified.
GAP 2: UDTs are not consistently used as part of the routine risk assessment for patients on opioids.
• RECOMMENDATION 2A: Use UDTs as part of the risk assessment tools prior to the initiation of opioid therapy and as a tool for reevaluating risk, using the clinical judgment of the treatment team.
• RECOMMENDATION 2B: Clinicians should educate patients on the use of UDTs and their role in identifying both appropriate and potentially inappropriate use" (PMFT, 2019).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTS are not approved or cleared by the U.S. Food and Drug Administration; however, FDA clearance or approval is not currently required by clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
80305	Drug tests(s), presumptive, any number of drug classes; any number of devices or procedures, (e.g., immunoassay) capable of being read by direct optical observation only (e.g., utilizing immunoassay [e.g., dipsticks, cups, cards, cartridges]), includes sample validation when performed, per date of service
80306	Drug tests(s), presumptive, any number of drug classes; any number of devices or procedures read by instrument-assisted direct optical observation (e.g., utilizing

	immunoassay [e.g., dipsticks, cups, cards, cartridges]), includes sample validation when performed, per date of service
80307	Drug test(s), presumptive, any number of drug classes, any number of devices or procedures; by instrument chemistry analyzers (e.g., utilizing immunoassay [e.g., EIA, ELISA, EMIT, FPIA, IA, KIMS, RIA]), chromatography (e.g., GC, HPLC), and mass spectrometry either with or without chromatography, (e.g., DART, DESI, GC-MS, GC-MS/MS, LC-MS, LC-MS/MS, LDTD, MALDI, TOF) includes sample validation when performed, per date of service
G0480	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 1-7 drug class(es), including metabolite(s) if performed
G0481	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 8-14 drug class(es), including metabolite(s) if performed
G0482	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 15-21 drug class(es), including metabolite(s) if performed
G0483	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA,

	ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 22 or more drug class(es), including metabolite(s) if performed
G0659	Drug test(s), definitive, utilizing drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem), excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase), performed without method or drug-specific calibration, without matrix-matched quality control material, or without use of stable isotope or other universally recognized internal standard(s) for each drug, drug metabolite or drug class per specimen; qualitative or quantitative, all sources, includes specimen validity testing, per day, any number of drug classes
0007U	Drug test(s), presumptive, with definitive confirmation of positive results, any number of drug classes, urine, includes specimen verification including DNA authentication in comparison to buccal DNA, per date of service Proprietary test: ToxProtect Lab/Manufacturer: Genotox Laboratories LTD
0011U	Prescription drug monitoring, evaluation of drugs present by LC-MS/MS, using oral fluid, reported as a comparison to an estimated steady-state range, per date of service including all drug compounds and metabolites Proprietary test: Cordant CORE™ Lab/Manufacturer: Cordant Health Solutions
0051U	Prescription drug monitoring, evaluation of drugs present by LC-MS/MS, urine, 31 drug panel, reported as quantitative results, detected or not detected, per date of service Proprietary test: UCompliDx Lab/Manufacturer: Elite Medical Laboratory Solutions, LLC (LDT)
0054U	Prescription drug monitoring, 14 or more classes of drugs and substances, definitive tandem mass spectrometry with chromatography, capillary blood, quantitative report with therapeutic and toxic ranges, including steady-state range for the prescribed dose when detected, per date of service Proprietary test: AssuranceRx Micro Serum Lab/Manufacturer: Firstox Laboratories, LLC
0082U	Drug Test(S), Definitive, 90 or More Drugs or Substances, Definitive Chromatography with Mass Spectrometry, and Presumptive, any Number of Drug Classes, by Instrument Chemistry Analyzer (Utilizing Immunoassay), Urine, Report of Presence or Absence of Each Drug, Drug Metabolite or Substance with Description and Severity of Significant Interactions Per Date of Service
0093U	Prescription drug monitoring, evaluation of 65 common drugs by LC-MS/MS, urine, each drug reported detected or not detected

	Proprietary test: ComplyRX Lab/Manufacturer: Claro Labs
0227U	Drug assay, presumptive, 30 or more drugs or metabolites, urine, liquid chromatography with tandem mass spectrometry (LC-MS/MS) using multiple reaction monitoring (MRM), with drug or metabolite description, includes sample validation Proprietary Test: Comprehensive Screen Lab/Manufacturer: Aspenti Health
0328U	Drug assay, definitive, 120 or more drugs and metabolites, urine, quantitative liquid chromatography with tandem mass spectrometry (LC-MS/MS), includes specimen validity and algorithmic analysis describing drug or metabolite and presence or absence of risks for a significant patient-adverse event, per date of service Proprietary test: CareView360 Lab/Manufacturer: Newstar Medical Laboratories, LLC

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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Updates, Gaps, Inconsistencies, and Recommendations

PAIN MANAGEMENT

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VIII. Revision History

Revision Date	Summary of Changes
4/27/22	The following modifications were made: Revised wording throughout coverage criteria for clarity; and updated coverage criteria #2 to read as follows: "Confirmatory/definitive qualitative or quantitative drug testing **MEETS COVERAGE CRITERIA**, up to seven drug classes, when laboratory-based definitive drug testing is specifically requested, the rationale is documented by the patient's treating physician, and ANY of the following conditions are met …"

Prostate Biopsies

Policy #: AHS – G2007	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 2/22/23 (See Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Prostate cancer is characterized by a malignancy of the small walnut-shaped gland that produces seminal fluid in males which ranges clinically from a microscopic, well-differentiated tumor that may never be clinically significant to an aggressive, high-grade cancer (Kantoff et al., 2020).

II. Related Policies

Policy Number	Policy Title
AHS-G2008	Prostate Cancer Screening
AHS-G2013	Hormonal Testing in Males
AHS-G2054	Liquid Biopsy
AHS-G2124	Serum Tumor Markers For Malignancies
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2166	Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. In the initial diagnosis of prostate cancer as a follow up to abnormal PSA results, presence of a palpable nodule on digital rectal examination, or suspicious radiologic findings, prostate biopsy involving 12 core extended sampling* (see Note 1 below) **MEETS COVERAGE CRITERIA.**

2. When the clinical suspicion of prostate cancer remains in an individual for whom an initial biopsy was negative for prostate cancer, follow-up biopsy (excluding prostate saturation biopsy) **MEETS COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3. Prostate saturation biopsy **DOES NOT MEET COVERAGE CRITERIA** for the diagnosis, staging, and management of prostate cancer.

*Note 1: One vial per sextant, with no more than two core samples per vial.

IV. Scientific Background

Prostate cancer is the most common cancer in American men and the second leading cause of death in men aged 65 years or older (Balducci et al., 1997; Tabayoyong & Abouassaly, 2015) with an estimated 191,930 new cases and 33,330 deaths in the US in 2020 (Siegel et al., 2020). About 11% of men will be diagnosed with prostate cancer during his lifetime (Kantoff et al., 2020).

Many cases of prostate cancer do not become clinically evident, as indicated in autopsy series, where prostate cancer is detected in approximately 30% of men age 55 and approximately 60% of men by age 80 (Bell et al., 2015). These data suggest that prostate cancer often grows so slowly that most men die of other causes before the disease becomes clinically advanced. Prostate cancer survival is related to many factors, especially the extent of tumor at the time of diagnosis. The five-year relative survival among men with cancer confined to the prostate (localized) or with just regional spread is 100%, compared with 31% among those diagnosed with distant metastases (Hoffman, 2022).

Findings on digital rectal examination (DRE) including the presence of nodules, induration, or asymmetry or elevated prostate specific antigen (PSA) levels indicate the need for prostate biopsy. Although generally considered safe, prostate biopsy is an invasive procedure and recommendations for its use are limited to a subset of patients. Screening the general population for prostate cancer remains a controversial issue (Hoffman, 2022). Screening may reduce the risk of distant-stage prostate cancer. The European Randomized Study of Screening for Prostate Cancer (ERSPC) enrolled 162,243 men ages 50 to 69 years. The cumulative incidence rate of metastatic disease in the regular screening group was 0.67 percent compared to the control group of 0.86 percent. The absolute risk reduction of metastatic disease was 3.1 per 1000 men randomized (Hoffman, 2022).

Multiple sampling schemes have been developed to improve the accuracy of prostate biopsy in the detection of cancer. Systematic prostate sampling is performed and augmented by additional sampling of any abnormal areas found on ultrasound or rectal examination (Gosselaar et al., 2008). During transrectal ultrasound (TRUS)-guided biopsy, a six-core, or sextant biopsy technique, takes one sample each from the apex, base, and mid-prostate on each side (Hodge et al., 1989). However, this method

may miss approximately 30% of clinically significant cancers and has been replaced by extended core biopsy which obtains five to seven evenly-distributed specimens from each side, sampling more extensively from the lateral aspects of the prostate (Benway & Andriole, 2021). A meta-analysis by Eichler et al found that schemes with 12 core samples that took additional laterally directed cores detected 31% more cancers compared with a six-core approach, with increasing number of cores significantly associated with increased detection of prostate cancer (Eichler et al., 2006). This biopsy method has been used to obtain up to 18 cores for evaluation (Benway & Andriole, 2021).

Saturation biopsy involves extensive sampling of the prostate, obtaining up to 24 core samples. Saturation biopsy is not appropriate for initial screening as it does not provide increased cancer detection when used for first-time biopsy but may provide increased sensitivity when repeat biopsies are performed and can be considered after one or more negative TRUS-biopsies. Saturation biopsy detects prostate cancer in approximately 22% to 33% of patients undergoing repeat biopsy, but it is associated with a higher incidence of complications (Benway & Andriole, 2021).

Several complications may occur with biopsy. Firstly, the samples from a biopsy may be inadequate to make a diagnosis; the cores obtained may not be of high enough quality or more cores may be needed. Other findings such as an abnormal but nonmalignant histology may warrant a repeat biopsy. Clinical complications such as inflammation, bleeding, infection, and urinary obstruction are also possible (Benway & Andriole, 2021). Pepe et al. estimated the rate of clinical complication after a transperineal biopsy to be as high as 40% (Pepe & Aragona, 2007).

Clinical Utility and Validity

Thompson et al. (2015) studied whether saturation or transperineal biopsy altered oncological outcomes as compared with standard transrectal biopsy. 650 men were analyzed, and saturation biopsy was associated with "increased objective biopsy progression requiring treatment" on both the Kaplan-Meier analysis and multivariate Cox analysis. A logistic regression analysis of 179 men undergoing a radical prostatectomy (RP) found that transperineal biopsy was associated with lower likelihood of "unfavourable" RP pathology. The authors concluded that "saturation biopsy increased progression to treatment on AS; longer follow-up is needed to determine if this represents beneficial earlier detection of significant disease or over-treatment. Transperineal biopsy reduced the likelihood of unfavourable disease at RP, possibly due to earlier detection of anterior tumours" (Thompson et al., 2015).

Zaytoun et al. (2011) "compared saturation and extended repeat biopsy protocols after initially negative biopsy." 1056 men were included, with 393 men undergoing a 12-14 core biopsy ("extended") and 663 men undergoing a 20-24 core biopsy ("saturated"). Overall, prostate cancer was detected in 315 patients, but saturated biopsy detected a third more cancers and identified more cancers in a benign initial biopsy. 119 biopsies identified clinically "insignificant" cancer. The authors concluded, "Compared to extended biopsy, office-based saturation biopsy significantly increases cancer detection on repeat biopsy. The potential for increased detection of clinically insignificant cancer should be weighed against missing significant cases" (Zaytoun et al., 2011).

The Prostate Magnetic Resonance Imaging Study (PROMIS) study (Brown et al., 2018) "assessed the ability of multi-parametric MRI (mpMRI) to identify men who can safely avoid unnecessary biopsy" and compared mpMRI to TRUS-guided biopsy. A TPM-biopsy was included for comparison, and 576 men underwent all three tests. Clinically significant (CS) cancer was defined as "a Gleason score of ≥ 4 + 3

and/or cancer core length of ≥ 6 mm". For CS cancer, TRUS-guided biopsy showed a sensitivity of 48%, specificity of 96%, PPV of 90%, and NPV of 74%. The sensitivity of mpMRI was 93%, specificity was 41%, PPV was 51%, and NPV was 89%. A negative mpMRI scan was recorded for 158 men (27%). Of these, 17 were found to have CS cancer on TPM-biopsy. The authors also found that the most cost-effective strategy "involved testing all men with mpMRI, followed by MRI-guided TRUS-guided biopsy in those patients with suspected CS cancer, followed by rebiopsy if CS cancer was not detected" (Brown et al., 2018).

Sidana et al. compared the yield of MRI fusion biopsy (FBx) to 12-core TRUS biopsy (SBx) in patients with prior negative biopsies. 779 patients were included, and a total of 346 cancers were detected with 239 of 346 considered clinically significant. FBx diagnosed a total of 205 patients with SBx diagnosing an additional 34 patients. FBx identified high proportions of clinically significant cancers over all amounts of prior negative biopsies. The authors stated that "SBx added a relatively small diagnostic value to FBx for detecting CS disease" and concluded that "repeat SBx alone in patients with multiple prior negative biopsies will be hindered by lower yield and FBx should be utilized concurrently in these patients" (Sidana et al., 2018).

Pepe et al. investigated the diagnostic accuracies for clinically significant prostate cancer, multiparametric magnetic resonance imaging (MRI) and transperineal saturation prostate biopsy. Lesions with PI-RADS (Prostate Imaging Reporting and Data System) scores of 3 or higher were subjected to additional targeted fusion prostate biopsy. 1032 patients were included, with 372 deemed to have T1c prostate cancer. Further, 272 of these cases were considered "clinically significant". Saturation biopsy missed 12 of 272 clinically significant cancers, and targeted fusion prostate biopsy with the score cutoff of 3 missed 44 cases. However, the authors noted that using multiparametric MRI in combination with a score cutoff of 3 in PI-RADS would have prevented 49.3% of biopsies, and a score cut-off of 4 would have prevented 73.6% of biopsies, although the score cutoff of 4 missed 108 of 272 clinically significant cases. The authors concluded that multiparametric MRI could "significantly reduce the number of unnecessary repeat prostate biopsies in about 50% of cases in which a PI-RADS score of 3 or greater is used" (Pepe et al., 2018).

Pepe et al. investigated the amount of cores (combined with multiparametric MRI [mpMRI]) needed to diagnose "all clinically significant cases of prostate cancer (csPCa) in men subject to transperineal saturation biopsy (SPBx; 30 cores)". 875 patients were included. Stage 1 prostate cancer was found in 306 of these patients, with 222 of these classified as "clinically significant". The initial 20 needle cores obtained from SPBx identified all 222 cases of clinically significant prostate cancer, although it missed 84 of 129 indolent cases. Overall, the "diagnostic accuracy, sensitivity, and specificity [were] equal to 83.1%, 100%, and 65.1%, respectively." The authors concluded that "in men subject to mpMRI and/or TPBx, a maximum of 20 systematic transperineal needle cores detected all cases of csPCa and minimized the diagnosis of indolent cancers" (Pepe et al., 2020).

V. Guidelines and Recommendations

The American Urological Association (AUA)

The AUA published a paper (2015) on Optimal Techniques of Prostate Biopsy and Specimen Handling which recommended: "12-core systematic sampling methodology that incorporates apical and far-lateral cores in the template distribution. The results of our literature review suggest that collecting

more than 12 cores or sampling the transition zone offer no benefit for initial diagnostic biopsies. However, such approaches might be useful for resampling following a negative biopsy".

The AUA / American Society for Radiation Oncology (ASTRO) / Society of Urologic Oncology (SUO) published guidelines (Sanda et al., 2018), which state:

- "Localized prostate cancer patients who elect active surveillance should have accurate disease staging including systematic biopsy with ultrasound or MRI-guided imaging."
- "Localized prostate cancer patients undergoing active surveillance should be encouraged to have a confirmatory biopsy within the initial two years and surveillance biopsies thereafter."

In 2018, the American Society of Clinical Oncology (ASCO) endorsed the above 2017 AUA/ASTRO/SUO joint guideline, with only a minor disagreement on two cryosurgery recommendations (Bekelman et al., 2018).

National Comprehensive Cancer Network (NCCN)

NCCN Guidelines on Early Detection for Prostate Cancer (NCCN, 2019) state that "systematic prostate biopsy under TRUS guidance with or without targeted of lesions seen on pre-biopsy MRI is the recommended technique for prostate biopsy." It recommends the use of an extended pattern at least 12 core biopsies as it has been validated and results in enhances cancer detection compared to sextant biopsy schemes. Moreover, the NCCN states,

- "Anteriorly directed biopsy is not supported (NCCN, 2022a, 2022b) in routine biopsy. However, this can be added to an extended biopsy protocol in a repeat biopsy if PSA is persistently elevated".
- "A negative biopsy does not preclude a diagnosis of prostate cancer on subsequent biopsy. If clinical suspicion of cancer persists after a negative biopsy, consideration can be given to saturation biopsy strategies and/or the use of multiparametric MRI followed by an appropriate targeted biopsy technique based on the results."
- "Based on emerging evidence, the panel believes that a saturation biopsy strategy can be considered for very high-risk men with previous negative biopsies".
- "After 1 or more negative TRUS biopsies, men who are considered high-risk (e.g. those with persistently elevated or rising PSA) can be considered for MRI followed by targeted biopsy". The NCCN notes that targeted biopsy techniques include "cognitive or visual targeting, TRUS-MRI fusion platforms, and direct in-bore magnetic resonance biopsy-guided biopsy.
- "Overall, the panel believes that targeted biopsy techniques may help regions of cancer missed on prior biopsies and should be strongly considered in patients with a prior negative biopsy and persistent concern for cancer".
- "The panel believes that MRI-guided targeted biopsies can be considered in place of standard 12-core TRUS biopsies in initial biopsy setting…however…more information is needed about the generalizability of the findings of the trials mentioned above".

The NCCN also addressed prostate biopsy in their Prostate Cancer guideline. The NCCN remarks that biopsy (and/or multiparametric MRI) can be considered for active surveillance for patients with over 10 years life expectancy. The NCCN also states that a prostate biopsy should not be repeated "no more often than 12 months" unless clinically indicated (such as PSA increase). Finally, the NCCN states that a repeat biopsy can be indicated within 6 months "if the initial biopsy was less than 10 cores, or if assessment results show discordance" (NCCN, 2022a).

American College of Radiology (ACR)

The ACR (Coakley et al., 2017) rated TRUS guided biopsy a 9, and MRI targeted prostate biopsy a 7 in the most recent ACR Appropriateness Criteria for Prostate Cancer Pretreatment Detection, Surveillance and Staging for "clinically suspected prostate cancer with no prior biopsy". A rating of 7, 8 or 9 are usually appropriate. MRI targeted biopsy was rated an 8 and repeat TRUS biopsy rated a 7 in "clinically suspected prostate cancer, prior negative TRUS biopsy" as well as "clinically established low risk prostate cancer for active surveillance".

They note that "Overall, the clinical paradigm for prostate cancer diagnosis is rapidly moving towards MRI-targeted transrectal biopsy, based on substantial evidence from several centers (notably the National Institutes of Health; New York University [NYU]; University of California, Los Angeles [UCLA]; and Nijmegen) that this approach can transform baseline cancer evaluation when compared with traditional systematic biopsy, with fewer false negatives, better tumor characterization, improved tumor localization, and better treatment stratification, especially stratification to lower-risk cohorts that may be appropriate for active surveillance or focal therapy" (Coakley et al., 2017).

American Cancer Society (ACS)

The ACS published guidelines (Wolf et al., 2010), which state:

- "A PSA level of 4.0 ng/mL or greater historically has been used to recommend referral for further evaluation or biopsy, which remains a reasonable approach for men at average risk for prostate cancer."
  "For PSA levels between 2.5 ng/mL and 4.0 ng/mL, health care providers should consider an individualized risk assessment that incorporates other risk factors for prostate cancer, particularly for high-grade cancer, that may be used to recommend a biopsy. Factors that increase the risk of prostate cancer include African American race, family history of prostate cancer, increasing age, and abnormal DRE. A previous negative biopsy lowers the risk. Methods are available that merge this information to achieve an estimate of a man's overall risk of prostate cancer and, more specifically, of his risk of high-grade prostate cancer."

According to the ACS, an update to the guidelines for prostate cancer was initiated in 2019 (Smith et al., 2019).

US Preventive Services Task Force (USPSTF)

Within the 2018 USPSTF recommendation statement regarding prostate screening, they state, "Men with a positive PSA test result may undergo a transrectal ultrasound-guided core-needle biopsy of the prostate to diagnose prostate cancer… Although protocols vary, active surveillance usually includes regular, repeated PSA testing and often repeated digital rectal examination and prostate biopsy, with potential for exposure to repeated harms from biopsies" (USPSTF, 2018).

European Society for Medical Oncology (ESMO)

ESMO includes some recommendations for prostate biopsies:

- "Transperineal biopsies are recommended, rather than transrectal ultrasound (TRUS)-guided biopsies". ESMO further noted that "Targeted transperineal biopsies, in comparison with systematic

transrectal biopsies, result in an increased detection rate of clinically significant prostate cancer, a decreased detection rate of clinically insignificant prostate cancer, and fewer adverse events".

- When multiparametric MRI is positive (defined as [PI-RADS] ≥3), ESMO recommends performing a targeted (systematic or non-systematic) biopsy. However, when multiparametric MRI is negative (PI-RADS ≤2) and clinical suspicion of cancer is low, the biopsy can be omitted (Parker et al., 2020).

European Association of Urology

The EAU's recommendations on prostate biopsy include the following:

- The need for biopsy is based on PSA level or suspicious DRE/imaging, although limited PSA elevation alone should not prompt biopsy.
- "Ultrasound (US)-guided biopsy is now the standard of care…transurethral resection of the prostate should not be used as a tool for cancer detection".
- "Systematic biopsy is an acceptable approach in case mpMRI [multiparametric MRI] is unavailable".
- "Sextant biopsy is no longer considered adequate. At least eight systematic [core] biopsies are recommended in prostates with a size of about 30 cc. Ten to twelve core biopsies are recommended in larger prostates, with > twelve cores not being significantly more conclusive" (EAU, 2022).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA has cleared numerous devices including needles, reagents, instrumentation, and imaging systems for use in prostate biopsy. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
88305	Level IV – Surgical pathology, gross and microscopic examination
G0416	Surgical pathology, gross and microscopic examinations, for prostate needle biopsy, any method

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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Prostate Specific Antigen (PSA) Testing

Policy #: AHS – G2008	**Prior Policy Name & Number (as applicable):** G2008 – Prostate Cancer Screening
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Prostate-specific antigen (PSA) is a glycoprotein that is produced by both normal and neoplastic prostate tissue. In normal conditions, PSA is produced as a proenzyme in the prostate and secreted into the lumen. The propeptide is removed to activate the proenzyme; from there, it undergoes proteolysis to inactivate it. This inactive form may enter the bloodstream and circulate as "free" PSA. This process differs in prostate cancer; the basal cells that normally regulate this activation process are missing, which allows the secreted PSA direct access into the bloodstream. This increases the PSA concentration in the serum (Freedland, 2022).

Due to these reasons, PSA is often used in assessment of prostate cancer, such as screening, monitoring, diagnosis, and treatment management.

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For average-risk individuals 45-75 years of age, screening for prostate cancer with the total prostate-specific antigen (PSA) test **MEETS COVERAGE CRITERIA**.

2. For individuals 40-75 years of age, annual screening for prostate cancer with the total (PSA) test **MEETS COVERAGE CRITERIA** when one of the following conditions is met:
   a. Individual is of African ancestry.
   b. Individual has germline mutations that increase risk for prostate cancer.
   c. Individual has a suspicious family history.
3. For individuals over 75 years of age, who have little or no comorbidities (see Note 1), screening for prostate cancer with a total PSA test **MEETS COVERAGE CRITERIA**.
4. For individuals with previous total PSA results, repeat screening for prostate cancer with a total PSA test **MEETS COVERAGE CRITERIA** with the following frequency:
   a. For individuals less than 76 years of age, when total PSA <1 ng/ml and digital rectal exam (DRE) is normal (if done): Repeat screening at 2- to 4-year intervals
   b. For individuals less than 76 years of age, when total PSA is 1-3 ng/ml and DRE is normal (if done): Repeat screening at 1- to 2-year intervals
   c. For individuals greater than 75 years of age, when total PSA is < 4 ng/ml and DRE is normal (if done) and no other indications for biopsy: Repeat screening in select patients (very healthy individuals with little or no comorbidity) at 1- to 4- year intervals. and/or very suspicious DRE: Any one of the following **MEETS COVERAGE CRITERIA.**
5. TRUS-guided biopsy, percent free PSA, or a follow-up in 6-12 months with total PSA or DRE **MEETS COVERAGE CRITERIA** when any of the following conditions are met:
   a. For individuals less than 76 years of age, with a total PSA > 3mg/ml and/or a very suspicious DRE.
   b. For select individuals greater than 75 years of age (very healthy individuals with little or no comorbidity) with a total PSA > 4ng/ml or a very suspicious DRE.
6. For individuals thought to be at a higher risk despite at least one prior negative prostate biopsy, follow-up testing with percent free PSA **MEETS COVERAGE CRITERIA.**
7. Total PSA testing **MEETS COVERAGE CRITERIA** in any of the following situations:
   a) For initial prostate cancer diagnosis in individuals with signs and symptoms of prostate cancer (see Note 2).
   b) For follow-up of individuals with a current or previous diagnosis of prostate cancer.
   c) For ongoing monitoring of individuals who have undergone tumor resection or prostatectomy.
   d) For monitoring response to therapy.
   e) For detecting disease recurrence.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

8. The following testing **DOES NOT MEET COVERAGE CRITERIA**:

a. Percent free PSA as a first-line screening test for prostate cancer

b. Percent free PSA, free-to-total PSA ratio, and/or complexed PSA tests for the routine screening of prostate cancer.

NOTES:

NOTE 1: According to the NCCN guidelines, "Testing after 75 years of age should be done only in very healthy men with little or no comorbidity (especially if they have never undergone PSA testing or have a rising PSA) to detect the small number of aggressive cancers that pose a significant risk if left undetected until signs or symptoms develop. Widespread screening in this population would substantially increase rates of overdetection and is not recommended (NCCN, 2023b)." Additionally, the term individuals in this policy apply to individuals who have a prostate or were born with a prostate.

NOTE 2: According to ACS, 2019: "Most prostate cancers are found early, through screening. Early prostate cancer usually causes no symptoms. More advanced prostate cancers can sometimes cause symptoms, such as:

- Problems urinating, including a slow or weak urinary stream or the need to urinate more often, especially at night
- Blood in the urine or semen
- Trouble getting an erection (erectile dysfunction or ED)
- Pain in the hips, back (spine), chest (ribs), or other areas from cancer that has spread to bones
- Weakness or numbness in the legs or feet, or even loss of bladder or bowel control from cancer pressing on the spinal cord (ACS, 2019)."

III. Scientific Background

Prostate cancer is the most common cancer in American men and the second leading cause of death in men over 65 (Balducci et al., 1997; Tabayoyong & Abouassaly, 2015). According to the CDC (2022a), more than 204,000 prostate cancer cases are reported annually in the United States, leading to more than 31,000 prostate cancer deaths each year. The American Cancer Society estimates over 288,000 new cases and 34,000 deaths of prostate cancer in 2020 (American_Cancer_Society, 2020). Prostate cancer survival is related to many factors, especially the extent of the tumor at the time of diagnosis. The 5-year survival rate for men with localized or regional prostate cancer is nearly 100%, while the 5-year survival rate for men with distant prostate cancer, where the cancer has spread to other parts of the body such as the lungs, liver or bones, is 31% (ACS, 2022; Hoffman, 2023). About one man in nine will be diagnosed with prostate cancer during his lifetime in the United States (American_Cancer_Society, 2020).

Many cases of prostate cancer do not become clinically evident, as indicated in autopsy studies, where prostate cancer is detected in approximately 30 percent of men age 55 or older and approximately 60 percent of men by age 80 (K. J. Bell, Del Mar, Wright, Dickinson, & Glasziou, 2015). These data suggest that prostate cancer often grows so slowly that most men die of other causes before the disease becomes clinically advanced (Hoffman, 2023).

Most prostate cancers use androgen-dependent signaling for development and progression (Fisher et al., 2015). As the number of targeted therapy agents increase, it is crucial to determine which patients will benefit from these interventions. Understanding the molecular pathology will allow clinicians to provide better patient management. Recent studies have led to the classification of prostate cancer into different subtypes, yet the utility of this in the clinical setting is to be determined (Rodrigues, Butler, Estelles, & de Bono, 2014).

Prostate-specific antigen (PSA), a glycoprotein produced by prostate epithelial cells, is the most widely accepted biomarker for prostate cancer screening. Levels of this protein can be identified via a simple blood test; many doctors consider abnormal PSA levels to be above 4.0 ng/mL, although there is no official standardized normal or abnormal PSA level (NCI, 2022a). Further, PSA levels tend to increase with age, suggesting that age-specific PSA reference ranges may be important for clinical use (NCI, 2022a).

In serum, PSA can be identified in three forms. The main form is PSA bound by alpha-1 antichymotrypsin and accounts for approximately 75% of total PSA; PSA bound to alpha-2 macroglobulin has also been identified but cannot be detected by commercial immunoassays and represents less than 0.1% of PSA (Prcic, Begic, & Hiros, 2016). Finally, unbound or free PSA, which is the enzymatically inactive form, can be found in 5-50% of serum samples (Prcic et al., 2016). Total PSA measures the amount of all PSA identified in a sample. Some researchers claim that the amount of total versus free PSA in a sample can foreshadow prostate cancer risk (Prcic et al., 2016). Coban et al. (2016) reported that, while total PSA levels are an important prognostic factor for predicting prostate volumes, free PSA levels had a higher predictive value.

Analytical Validity

Prostate-specific antigen (PSA) was originally introduced as a tumor marker to detect cancer recurrence or disease progression following treatment (Brawley et al., 2018). It has become widely adopted for early detection of prostate cancer screening; however, its clinical utility in screening is controversial, and guidelines for PSA screening are conflicting. Non-optimal screening and treatment practices, including excessive screening among older men with lower life expectancy or comorbidities and overtreatment of men with low risk tumors, have contributed to treatment-related harm and a lower quality of life (Fleshner, Carlsson, & Roobol, 2017). Evidence is currently lacking to show that PSA screening actually saves lives; instead, it may only cause overdiagnosis and lead to complications in the treatment of indolent diseases (Ilic et al., 2018).

As PSA is not a cancer-specific marker, it causes many false results that conflict with other screening methods, such as the digital rectal examination (DRE) (Saini, 2016). For example, PSA may be elevated due to conditions including benign prostatic hyperplasia (BPH) or prostatitis. This is particularly

important as BPH is common among men over 50, the most common age group in which prostate cancer is observed. A study performed by Stimac et al. (2014) found PSA levels to be unusual despite testing negative for cancer. The authors concluded that subclinical inflammation had a major influence on free PSA levels only if the total levels were <10 ng/mL, and further note that clinical and acute inflammation produce a different profile of PSA release compared to a subclinical inflammation. Overall, the authors state that the molecular cause of the inflammation's changes to PSA forms are still unknown (Stimac et al., 2014). Furthermore, serum PSA is directly tied to the size of the prostate, which increases with age. Older men may see an increased concentration of PSA despite being completely healthy (Freedland, 2022; Stimac et al., 2014). Other factors such as medication can also affect PSA levels. Common medications, including statins, NSAIDs, acetaminophen, 5-alpha-reductase inhibitors, and thiazides, were all found to reduce PSA levels by varying degrees (Chang, Harshman, & Presti, 2010; Hamilton, Goldberg, Platz, & Freedland, 2008; Singer, Palapattu, & van Wijngaarden, 2008; Wang, Liu, Kreis, & Budman, 1997).

Clinical Utility

The utility of PSA-based screening is also in question. A randomized clinical trial focusing on men undergoing a single PSA-based screening (n = 189386) compared to controls not undergoing a PSA-based screening (n = 219439) found no difference in cancer mortality after a median follow-up of 10 years. The mortality rate in 1,000 individuals was 0.30 in the intervention group compared to 0.31 in the control group, or one extra death per 100,000 patients. Although prostate cancer was diagnosed more often in the intervention group (4.3% compared to 3.6% in the control group), the mortality rate was almost identical between both groups (Martin et al., 2018).

A systematic review and meta-analysis of 341,342 patients evaluated the overall effectiveness of prostate cancer screening. Results from this study showed that while PSA screening did lead to an increase in the identification of prostate cancer cases at all stages, it did not necessarily reduce the amount of overall or disease specific mortality rates (Ilic et al., 2018). This highlights the uncertainty regarding the effectiveness of prostate cancer screening. The authors also noted that "PSA screening is associated with considerable biopsy-related and cancer treatment-related complications (Ilic et al., 2018)."

In May of 2012, the USPSTF released a grade D recommendation against PSA prostate cancer screening (Ahlering et al., 2019). In 2018, the recommendation was switched to a grade C recommendation, now suggesting that men ages 55 to 69 could be screened for prostate cancer if first counseled about the benefits and harms of screening (USPSTF, 2018). Nonetheless, when the grade D recommendation was first released, many researchers were worried that an increase in late stage prostate cancer cases would be identified, leading to greater rates of prostate cancer-specific mortality. To assess this risk, data from a total of 19,602 patients from nine high volume referral centers in the United States was collected and analyzed during the time that the USPSTF grade D recommendation was in effect. The researchers found that "All centers experienced consistent decreases of low-grade disease and absolute increases in intermediate and high-risk cancer. For any given age and PSA, propensity matching demonstrates more aggressive disease in the post-recommendation era (Ahlering et al., 2019)."

Osses, et al. (2019) assessed the results of 1,134 men screened for prostate cancer in a 19 year follow up study; at the start of the study, all men were between the ages of 55 and 74. Unfortunately, 63% of the

cohort was deceased by the 19-year follow-up period for various reasons. Still, the researchers noted that results suggested "a more substantial reduction in metastatic disease and cancer-specific mortality in favor of prostate cancer screening than previously reported (Osses et al., 2019)." However, more research needs to be completed with a larger sample size to confirm this conclusion.

Magnani et al. (2021) performed a cost analysis on "first-line prostate cancer management" using real-world data. A total of 3433 patients were included, and outcomes such as active surveillance (AS), surgery, and radiation were considered. Surgery was found to be the most common option, with 54.6% of the cohort compared to 22.3% for radiation and 23% for AS. Over a period of two years following diagnosis, AS was found to be the cheapest option at $2.97/day (d), with surgery costing $5.67/d and radiation costing $9.34/d, for "favorable" disease. For "unfavorable" disease, surgery cost $7.17/d and radiation cost $16.34/d. Over a period of five years following diagnosis, AS was found to be cheaper than surgery, by an amount of $2.71/d to $2.87 for surgery and $4.36 for favorable disease. For unfavorable disease, surgery remained cheaper than radiation, by an amount of $4.15/d to $10.32/d. The authors did remark that this information came from a single health care system and were based on benchmark Medicare estimates rather than actual payment exchanges (Magnani et al., 2021).

Baniak et al. (2020) compared the clinicopathologic and molecular characteristics of prostate cancer in 90 younger men (45 years or younger) to 200 men of typical screening age (60-65 years). The authors found that younger men tended to have lower PSA values, but a higher frequency of family history of prostate cancer. No significant differences were found in staging or pathological characteristics of core biopsy specimens between the two groups. The younger cohort was also found to have a higher frequency of "grade group 1 disease" at radical prostatectomy. Finally, no statistically significant differences were found regarding prostatic adenocarcinoma (PCa)-specific recurrence/progression or death between the two cohorts (Baniak et al., 2020).

IV. Guidelines and Recommendations

The American Association of Family Physicians (AAFP)

The AAFP recommends against the use of PSA-based testing for prostate cancer screening. For men between 55 to 69 years of age who are considering prostate cancer screening, the physician should discuss the risks and benefits and engage in shared decision making before undergoing the screening process. In addition, the AAFP recommends against prostate cancer screening in men older than 70 (AAFP, 2018b).

The American Academy of Family Physicians (AAFP)

The AAFP, with Choosing Wisely, have published guidelines on prostate cancer. These guidelines state that screening may prevent mortality, but "Whether this potentially small benefit in mortality outweighs the potential harms is dependent on the values and preferences of individual men. Therefore, for men who express a desire for prostate cancer screening, it should only be performed following a discussion of the potential benefits and harms. Routine screening for prostate cancer should not be done. PSA-based prostate cancer screening should not be performed in men over 70 years of age (AAFP, 2018a)."

The United States Preventive Services Task Force (USPSTF, 2018)

The USPSTF issued additional draft guidelines which recommend that clinicians inform men ages 55 to 69 years about the potential benefits and harms of PSA–based screening for prostate cancer, noting that the decision to be screened should be up to the patient. The USPSTF also states that screening offers a small potential benefit of reducing the chance of dying of prostate cancer. However, many men may be harmed due to false positives and its side effects such as overdiagnosis or other complications such as impotence. The USPSTF recommends discussion with a clinician before deciding to screen, ultimately giving this screening a "C" recommendation. Furthermore, the USPSTF recommends against PSA-based screening for prostate cancer in men over 70 (USPSTF, 2018). The CDC follows the USPSTF recommendations as well (CDC, 2022b).

The Memorial Sloan Kettering Cancer Center

The Memorial Sloan Kettering Cancer Center issued screening guidelines on prostate cancer based on the individual's age. Their guidelines are for men who are expected to live at least ten years. "Men ages 45 to 49 should have a baseline PSA test. If the PSA level is 3 ng / mL or higher, men should talk with their doctor about having a biopsy of the prostate. If the PSA level is between 1 and 3 ng / mL, men should see their doctor for another PSA test every two to four years. If the PSA level is less than 1 ng / mL, men should see their doctor for another PSA test between the ages of 51 and 55. Men ages 50 to 59 should have their PSA level checked. If the PSA level is 3 ng / mL or higher, men should talk with their doctor about having a biopsy of the prostate. If the PSA level is between 1 and 3 ng / mL, men should see their doctor for another PSA test every two to four years. If the PSA level is less than 1 ng / mL, men should see their doctor for another PSA test at age 60. Men ages 60 to 70 should have their PSA level checked. If the PSA level is 3 ng / mL or higher, men should talk with their doctor about having a biopsy of the prostate. If the PSA level is between 1 and 3 ng / mL, men should see their doctor for another PSA test every two to four years. If the PSA level is less than 1 ng / mL, no further screening is recommended. Men ages 71 to 75 should talk with their doctor about whether to have a PSA test. This decision should be based on past PSA levels and the health of the man. Prostate cancer screening is not recommended for men ages 76 or older. A high PSA level does not generally mean that a man should have a prostate biopsy" (Center, 2022).

The National Cancer Coalition Network (NCCN)

The NCCN also recommends that patients make informed decisions regarding enrollment in an early detection program. Factors such as personal history, previous testing, family history, and race should be considered for determination if and when an early detection protocol is implemented. The guidelines stated that most panel members favored informed testing starting at 45. The panel supports screening in men until 75, and then continuing screening only in very healthy patients with little or no comorbidity to detect the life threatening and aggressive cancers. However, widespread screening in this age group is not recommended (NCCN, 2023b). The NCCN also noted their concern about "the problems of overtreatment related to the increased frequency of diagnosis of prostate cancer from widespread use of PSA for early detection of screening" (NCCN, 2023a).

For initial testing, the NCCN recommends that "baseline PSA testing should be offered to healthy, well-informed men aged 45 to 75 years based on the results of RCTs (NCCN, 2023b)." The NCCN also recommends screening starting at 40 years for certain higher risk populations, such as those with

"African ancestry", "suspicious family history", and germline mutations that increase risk of prostate cancer. Further, baseline testing may be ordered along with a DRE, and any elevated levels should be double checked with repeat testing.

The NCCN considers three categories for "early evaluation detection"; men of average risk (45-75 years), men of increased risk (such as men with "African ancestry," "suspicious family history," and "germline mutations that increase the risk of prostate cancer" [such as BRCA]), and men above 75 years.

- For men aged 45 to 75 years, the panel recommends repeat testing every 2 to 4 years if PSA is <1 ng/mL and every 1 to 2 years if PSA is 1 to 3 ng/ml. If PSA > 3 ng/ml (or if the DRE is "very suspicious"), a biopsy should be considered.
- For high-risk populations, the above decision tree is identical for men of increased risk; the only difference is that NCCN recommends starting these evaluations at 40 years for the high-risk populations. The NCCN also writes to "consider" screening these high-risk populations "annually" rather than the less frequent intervals discussed.
- For men over 75 years, repeat testing in select patients at 1 – 4-year intervals is recommended if the PSA is <4 ng/ml, the DRE is normal, and there are no other indications for biopsy. If PSA >4 ng/ml, a repeat PSA test is recommended, followed-by a transrectal ultrasound (TRUS) or transperineal-guided biopsy.
- Regarding biopsies, the NCCN writes that a repeat PSA or other biomarkers that "improve specificity of screening" may be considered for evaluation. A follow-up 6-12 months after the biopsy to test for PSA may also be performed. If a biopsy is negative for cancer, the panel "recommends repeat PSA and DRE testing at 6- to 24-month intervals with consideration of repeat biopsy results." (NCCN, 2023b).

The NCCN also comments on several biomarkers' ability to assess early detection of prostate cancer.

- "cPSA", the alpha-1-antichemotrypsin complexed form of PSA, has been shown to provide information similar to the traditional free PSA to total PSA ratio. cPSA has been approved "as an aid in the detection of prostate cancer in men aged 50 or older in conjunction with DRE" but has not seen much clinical use as of time of writing.

The NCCN also includes recommendations for PSA testing in non-screening situations, such as monitoring. Regarding "patients initially treated with intent to cure", the NCCN recommends testing serum PSA levels "every 6 to 12 months for the first 5 years and then annually." The NCCN also notes that for men with "high" risk of recurrence, testing PSA every 3 months may be preferred. For patients with "castration-naïve disease on ADT [androgen deprivation therapy], PSA measurement may be done every 3-6 months (NCCN, 2023a).

The American Cancer Society (ACS)

The ACS recommends that physicians provide patients with information on benefits, risks, and uncertainties of the PSA test, and state that screening not be done until such information is received.

The ACS recommends that discussions (and screening) begin at age 50 for individuals of average risk, at

age 45 for those at increased risk, and at age 40 for those at highest risk (those with more than one first degree relative with a history of early-onset prostate cancer (ACS, 2021). After this discussion, indivudals who want to be screened should get the PSA screening and the digital rectal exam (DRE). Because prostate cancer is slow-growing, the ACS does not recommend PSA screening in any individual with a life expectancy of less than 10 years, regardless of age or family history. If the initial PSA test is in normal range, the ACS recommends different testing intervals based on the initial test. For patients with results less than 2.5 ng/mL, the screening interval should be 2 years. For patients with initial results is at or higher than 2.5 ng/mL, the screening interval should be annually (ACS, 2021).

The National Cancer Institute (NCI)

The NCI has deemed the evidence insufficient to determine whether PSA-based screening reduces mortality of prostate cancer. The NCI states that although screening can detect cancer in its earlier stages, it is unclear that earlier detection (and treatment) changes the natural course of the disease. The NCI also states that there is significant harm in screening such as overdiagnosis and complications caused by the screenings (NCI, 2022b).

The American College of Physicians (ACP)

The ACP agrees with the informed decision-making requirement for PSA testing, and states that clinicians should not screen using the PSA test in patients who "do not express a clear preference for screening." The ACP recommends that these discussions take place for men of average risk, ages 50 to 69 years. Finally, the ACP recommend against screening with PSA for individuals under 50 or over 70, and those with a life expectancy of less than 10 years (Qaseem et al., 2013; Wilt et al., 2015).

The American Urological Association (AUA)

The AUA recommends against use of PSA screening in men under 40, and routine screening for average-risk men between ages 40 to 54 years. The AUA does recommend informed decision making for men ages 55 to 69 years. The AUA recommends against PSA screening in men 70 years of age and older, or in any man with a life expectancy less than 10 – 15 years, although it is acknowledged that some men in excellent health 70 years and older may benefit from screening. The AUA recommends an individualized screening program be developed for individuals less than 55 years old, who are at high risk, such as those with a positive family history and African Americans. The AUA notes that a routine screening interval of two or more years may be preferred, but also notes that screening intervals "can be individualized by a baseline PSA level" (Carter et al., 2013).

The European Society for Medical Oncology (ESMO)

The ESMO recommends against population-based screening for prostate cancer because the reduction in mortality does not offset the harms done, such as overdiagnosis and overtreatment. Early PSA testing should only be offered to men > 50 years, men > 45 years with a family history of prostate

cancer, African Americans > 45 years, and *BRCA1/2* carriers who are > 40 years of age. Prostate cancer screening should not be performed in asymptomatic men with a life expectancy of less than ten years. ESMO also recommends against screening in asymptomatic men over 70 (Parker et al., 2020).

The American Association of Clinical Urologists Inc. (AACU)

The AACU recommends use of tissue-based molecular testing to assess risk stratification in prostate cancer treatment decision making. The AACU states pursing germline testing when appropriate is encouraged and support any further research into these tests. The Large Urology Group Practice Association (LUGPA) endorses this position statement by the AACU (AACU, 2018; LUGPA, 2018).

The European Association of Urology (EAU), European Society for Radiotherapy and Oncology (ESTRO) and International Society of Geriatric Oncology (SIOG)

Joint guidelines on prostate cancer screening and early detection from the EAU, ESTRO and SIOG include the table below taken from Mottet et al. (2020).

Recommendation	Strength rating
Do not subject men to prostate-specific antigen (PSA) testing without counselling them on the potential risks and benefits.	Strong
Offer an individualised risk-adapted strategy for early detection to a well-informed man with a good performance status (PS) and a life-expectancy of at least ten to fifteen years.	Weak
Offer early PSA testing in well-informed men at elevated risk of having PCa: men > 50 years of age; men > 45 years of age and a family history of PCa; African-Americans > 45 years of age. Men carrying *BRCA2* mutations > 40 years of age	Strong
Offer a risk-adapted strategy (based on initial PSA level), with follow-up intervals of two years for those initially at risk: men with a PSA level of > 1 ng/mL at 40 years of age; men with a PSA level of > 2 ng/mL at 60 years of age; Postpone follow-up to eight years in those not at risk.	Weak
Stop early diagnosis of PCa based on life expectancy and performance status; men who have a life-expectancy of < fifteen years are unlikely to benefit.	Strong

Additional guidelines for the risk-assessment of asymptomatic men from (Mottet et al., 2020) state:

Recommendation	Strength rating
To avoid unnecessary biopsies, offer further risk-assessment to asymptomatic men with a normal digital rectal examination (DRE) and a prostate-specific antigen (PSA) level between 2-10 ng/mL prior to performing a prostate biopsy. Use one of the following tools: risk-calculator; imaging; **an additional serum or urine-based test (weak strength rating)**	Strong

The guideline also notes the presence of newer biological markers, such as "TMPRSS2-ERG fusion, PCA3, or kallikreins as incorporated in the Phi or 4Kscore tests" but despite promising early results, the guideline considers these markers to have "too limited data to implement these markers into routine screening programmes." (Mottet et al., 2020)

V. Applicable State and Federal Regulations

The FDA has approved several screening tests for prostate cancer beginning with a PSA immunoassay in 1986 (FDA, 2023).

On June 14, 2012, the FDA approved the Access® Hybritech® p2PSA assay created by Beckman Coulter, Inc. From the FDA website: "The Access® Hybritech® p2PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of [-2] proPSA antigen, an isoform of free PSA, in human serum using the Access Immunoassay Systems. Access@ Hybritech® p2PSA is intended to be used in combination with Access® Hybritech® (total) PSA and Access@ Hybritech@ free PSA to calculate the Beckman Coulter Prostate Health Index (phi), an In Vitro Diagnostic Multivariate Index Assay (IVDMIA)" (FDA, 2012).

A search of the FDA database on 12/28/2020 using the term "PSA" yielded 97 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
84152	Prostate specific antigen (PSA); complexed (direct measurement)
84153	Prostate specific antigen (PSA); total
84154	Prostate specific antigen (PSA); free

G0103	Prostate cancer screening; prostate specific antigen test (PSA)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes

ST2 Assay for Chronic Heart Failure

Policy #: AHS – G2130	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Heart failure (HF) comprises a major cause of morbidity and mortality worldwide. HF is a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood (Yancy et al., 2013).

Suppression of tumorigenicity 2 (ST2) is a marker of cardiomyocyte stress and fibrosis that provides incremental value to natriuretic peptides for risk stratification of patients with a wide spectrum of cardiovascular diseases (Bayes-Genis, Zhang, & Ky, 2015).

II. Related Policies

Policy Number	Policy Title
AHS-G2050	Cardiovascular Disease Risk Assessment
AHS-G2150	Cardiac Biomarkers for Myocardial Infarction
AHS-M2025	Genetic Testing for Inherited Cardiomyopathies and Channelopathies

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

1. The use of the Presage® ST2 Assay to evaluate the prognosis of patients diagnosed with chronic heart failure **DOES NOT MEET COVERAGE CRITERIA**.
2. The use of the Presage® ST2 Assay to guide management (pharmacological, device-based, exercise, etc.) of patients diagnosed with chronic heart failure **DOES NOT MEET COVERAGE CRITERIA**.
3. The use of the Presage® ST2 Assay in the post cardiac transplantation period, including, but not limited to, predicting prognosis and predicting acute cellular rejection, **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

HF is a complex clinical syndrome resulting from any structural or functional impairment of ventricular filling or ejection of blood, including disorders of the pericardium, myocardium, endocardium, heart valves, great vessels, or certain metabolic abnormalities (W. Colucci, 2020). Most patients with HF have symptoms due to impaired left ventricular (LV) myocardial function (W. Colucci, Dunlay, Shannon, 2020; Yancy et al., 2013). The most common symptoms of HF are dyspnea and fatigue, which may limit exercise tolerance and fluid retention. Some patients have exercise intolerance but little evidence of fluid retention, whereas others complain primarily of edema, dyspnea, or fatigue (W. Colucci, Dunlay, Shannon, 2020). Heart failure is often a progressive condition, beginning with predisposing factors and leading to the development and worsening of clinical illness (Colucci, 2021; W. Colucci, Dunlay, Shannon, 2020).

No single diagnostic test for HF exists because it is largely a clinical diagnosis based on a careful history and physical examination. However, biomarkers of cardiovascular diseases have been developed for diagnosis and prognosis, and the use of several biomarkers is now considered the standard of care. ST2 is a marker of cardiomyocyte stress and fibrosis that adds additional value to natriuretic peptides, resulting in a risk stratification of patients with a wide spectrum of cardiovascular diseases (Bayes-Genis et al., 2015).

ST2 is part of the interleukin-1 receptor family with two isoforms, soluble ST2 (sST2) and ST2L. ST2 is the receptor of the IL-33 cytokine that can be secreted by living cells in response to cellular stress and mechanical strain. IL-33 binds the receptor complex of ST2L and IL-1R accessory protein and reduces myocardial fibrosis, prevents cardiomyocyte hypertrophy, reduces apoptosis, and improves myocardial function. The cardioprotective effects of IL-33 are specifically through the ST2L receptor. However, sST2 may also bind IL-33, blocking the interaction between IL-33/ST2L. This eliminates the cardioprotective effects of the IL-33/ST2L interaction (Pascual-Figal & Januzzi, 2015). Experimentally, this leads to cardiac hypertrophy, fibrosis, and ventricular dysfunction (Januzzi, Mebazaa, & Di Somma, 2015)

One of the main proprietary tests used to assess ST2 levels is the Presage Assay by Critical Diagnostics. This assay was approved by the FDA on December 9, 2011. According to the FDA, "The Critical Diagnostics Presage® ST2 Assay kit is an in vitro diagnostic device that quantitatively measures ST2 in serum or plasma by enzyme-linked immunosorbent assay (ELISA) in a microtiter plate format. The

Presage® ST2 Assay is indicated to be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure". The manufacturer claims a measuring range of 3.1 ng/mL of soluble ST2 to 200 ng/mL, and the data based on 1100 samples supports this claim. These 1100 samples found coefficient of variation of under 5%, a linear curve, and a r^2=0.99 (FDA, 2011).

Clinical Utility and Validity

Ky et al. conducted a multi-center prospective study to evaluate whether plasma ST2 levels predict adverse outcomes in 1,141 chronic heart failure outpatients. Patients in the highest ST2 tertile (ST2 > 36.3 ng/mL) had a "markedly increased" risk (hazard ratio 3.2) of adverse outcomes compared to the lowest tertile ≤22.3 ng/mL). The investigators concluded that "ST2 is a potent marker of risk in chronic heart failure and when used in combination with NT-proBNP offers moderate improvement in assessing prognosis beyond clinical risk scores" (Ky et al., 2011).

Broch et al. studied the association between sST2 and cause-specific outcome in 1449 patients enrolled in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA study). Soluble ST2 was measured in 1449 patients ≥60 years of age with left ventricular ejection fraction ≤40% due to ischemic heart disease. ST2 remained associated with death due to worsening HF, hospitalization due to worsening HF, and hospitalization due to any CV cause, even after full adjustment for N-terminal pro brain natriuretic peptide and C-reactive protein. A cut-off point of 15.5% increase of SST2 was associated with hospitalization, but not with any other outcome. This increase became weakly associated with both the primary endpoint (cardiovascular events such as stroke) and hospitalization. The investigators concluded that "Soluble ST2 is associated with adverse outcomes in older patients with systolic, ischemic HF. In particular, sST2 is independently associated with worsening HF (Broch et al., 2012).

Wang et al. studied the prognostic value of three novel biomarkers induced by cardiovascular stress. The investigators measured sST2, growth differentiation factor-15, and high-sensitivity troponin I in 3,428 participants in the Framingham Heart Study. Multivariable-adjusted proportional hazards models were performed to assess the individual and combined ability of the biomarkers to predict adverse outcomes. The three new biomarkers were associated with death, major cardiovascular events, and heart failure, but not with coronary events. The investigators concluded that the findings demonstrated the prognostic value of the newer biomarkers in apparently healthy individuals (Wang et al., 2012).

Felker et al. studied the association of ST2 level with long-term clinical outcomes in ambulatory heart failure patients enrolled in the HF-ACTION study – a multicenter, randomized study of exercise training in HF. ST2 was analyzed in a subset of 910 patients with evaluable plasma samples and correlations and Cox models were used to assess the relationship among ST2, functional capacity, and long-term outcomes. ST2 was "modestly" associated with measures of clinical capacity, such as death or hospitalization from HF but did not add to any reclassification of risk, improve discrimination of risk, or lead to any reclassification improvements" (Felker et al., 2013).

Anand et al. evaluated the association between soluble ST2 (sST2) and patient outcomes. sST2 was measured at baseline (n=1650), 4 months (n=1345), and 12 months (n=1094) in Valsartan Heart Failure

Trial. The authors observed that baseline sST2 (average of 28.7±16.2 ng/mL) was nonlinearly associated with patient outcomes. However, only sST2 levels <33.2 ng/mL were significantly related to patient outcomes when 23 other clinical variables were added to the regression model. The authors concluded that additional research is needed to determine whether monitoring ST2 levels can improve patient outcomes (Anand, Rector, Kuskowski, Snider, & Cohn, 2014).

Januzzi et al. conducted a retrospective study to assess sST2 as a prognostic marker after orthotopic heart transplantation (OHT) and as a test to predict acute cellular rejection. sST2 concentrations were measured in 241 patients following OHT. Elevated sST2 was associated with cellular rejection, with highest rates of cellular rejection in the 4th sST2 quartile. No significant association between sST2 and antibody-mediated rejection or allograft vasculopathy was found. A sST2 level of ≥ 30 ng/mL was found to independently predict death over the 7-year follow-up with a hazard ratio of 2.1. The investigators concluded that sST2 levels are associated with the presence of cellular rejection and predict long-term mortality following OHT (Januzzi et al., 2013).

Boman et al. assessed the prognostic value of ST2 on cardiovascular mortality. 159 patients were evaluated, but ST2 was not found to be significantly associated with cardiovascular mortality or all-cause mortality. Furthermore, no significant interaction of ST2 and N-terminal pro-hormone of brain natriuretic peptide /N-terminal pro-B-type natriuretic peptide was found (Boman, Thormark Frost, Bergman, & Olofsson, 2018).

Mueller et al. evaluated the prognostic and diagnostic value of sST2 (measured with Presage) for HF in an emergency setting (along with two other biomarkers, galectin-3 and BNP). 137 patients had dyspnea attributed to acute HF, and BNP was evaluated to have a 0.92 area under the curve (AUC) for the diagnosis of HF. For comparison, the AUC of sST2 was 0.63 and the AUC of galectin-3 was 0.57. Of these 137 patients, 41 died and 96 returned for follow-up. The AUC of BNP for the prediction of "all-cause mortality" was 0.72 similar to galectin-3 and sST2 (0.70 and 0.75, respectively). The authors concluded all three biomarkers to be approximately equally useful for prediction of all-cause mortality in patients with acute HF, but only BNP was found to be useful as a diagnostic aid in patients presenting with dyspnea (Mueller et al., 2016).

Stojkovic et al. (2018) published a study which concluded that GDF-15 is superior to sST2 in prediction of fatal arrhythmic events and all-cause mortality in dilated cardiomyopathy (DCM). 52 patients with DCM and left ventricular ejection fraction (LVEF) of under 50% were enrolled in the study, and only GDF-15 was found to be associated with increased risk of arrhythmic death or resuscitated cardiac arrest (hazard ratio: 2.1). Although ST2 was considered an independent predictor of "all-cause mortality", only GDF-15 was significantly associated with all-cause mortality when LVEF, ST2, GDF-15, and NYHA functional class were considered (Stojkovic et al., 2018).

Tyminska et al. investigated the association of galectin-3 and soluble ST2 (sST2) with the development of heart failure, including "echocardiographic parameters of HF [heart failure] (ejection fraction, atrial and ventricular size, left ventricular hypertrophy, e', and E/e') in patients with ST-segment elevation myocardial infarction (STEMI)…". 117 patients were included, and the primary endpoint was "HF onset at one year follow up". Mean baseline concentrations of sST2 (26.4 ng/mL) were increased at one-year

follow-up (31.4 ng/mL), and patients that reached the endpoint (n = 43) had a mean concentration of 33.1 ng/mL. Although sST2 was found to be a predictor of the primary endpoint in univariate logistic regression analysis, it was not significant in multivariate analysis (Tyminska et al., 2019).

Dimitropoulos et al. investigated the association of soluble suppression of tumorigenesis-2 (sST2) with endothelial function in patients with ischemic heart failure. A total of 143 patients with "table HF of ischemic etiology and reduced left ventricular ejection fraction (LVEF)" were included along with 77 controls. The authors found an increased level of sST2 in HF patients compared to controls (15.8 ng/mL compared to 12.5 ng/mL). Within the HF group, there was no association of LVEF with sST2. Overall, sST2 levels were found to be increased and associated with functional capacity in "patients with chronic HF of ischemic etiology". Finally, the authors found an inverse association between flow-mediated dilation and sST2 levels, which the authors stated "highlight[ed] the interplay between the dysfunctional endothelium and HF pathophysiologic mechanisms" (Dimitropoulos et al., 2020).

Hou et al. aimed to investigate the association between sST2 levels and clinical outcomes of high-risk heart failure. The primary endpoint was defined as all-cause mortality. A total of 150 patients were included; all-cause mortality occurred in 16 of the patients over the course follow-up. The authors found that all-cause mortality increased significantly above 34.98846 ng/mL by a factor of 16% to 5.33%. After adjusting the model for certain co-factors (age, gender, et al.), and after adding NT-proBNP, "the risk of all-cause death was increased by 2.5% and 1.9%, respectively, per ng/ml of sST2". The authors identified the best sST2 cut-off for predicting all-cause mortality to be 43.42671 ng/ml, with an area under the curve of 0.72, sensitivity of 0.69, and specificity of 0.69. Risk of all-cause mortality was found to be 21.2% above this cutoff and 5.1% below it, with a corresponding hazard ratio of 3.30. The authors concluded that "Patients with sST2 levels more than 43.42671 ng/ml even after ICD implantation should therefore be monitored carefully" (Hou et al., 2020).

V. Guidelines and Recommendations

American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA)

In 2017, the ACC, AHA, and HFSA jointly updated evidence-based guidelines for the management of heart failure (HF). The ACC/AHA/HFSA stated that "Biomarkers of myocardial fibrosis, soluble ST2 receptor, and galectin-3 are predictive of hospitalization and death and may provide incremental prognostic value over natriuretic peptide levels in patients with HF." The guidelines also note that "in patients with chronic HF, clinically available tests such as biomarkers of myocardial injury or fibrosis may be considered for additive risk stratification in patients" (Yancy Clyde et al., 2017).

American Heart Association (AHA)

The AHA notes sST2 as an "emerging" biomarker that supports diagnosis of HF with preserved ejection fraction, a biomarker that may predict mortality and HF events, and a biomarker that correlates with left ventricular end-diastolic pressure. The AHA states that sST2 has numerous advantages as a biomarker, namely its concentration being unaffected by BMI, age, or renal function. SST2 is stated to correlate with HF prognosis as well. Overall, AHA states that out of the newer biomarkers (SST2, ST2, Gal-3, and GDF-15), "most appeal is driven by sST2" (Chow Sheryl et al., 2017).

A Scientific Statement published in 2019 also considered ST2 as the most "promising clinically", but also mentioned the limitations in consistency and utility in most inflammatory mediators. The Statement notes several clinical studies focusing on sST2 that are in progress as of March 24, 2020 (Cresci et al., 2019).

Canadian Cardiovascular Society (CCS)

The CCS notes sST2 as a potential prognostic biomarker and states that "might be superior" to galectin-3. However, the CCS also remarks that it is "unclear" if using sST2 in HF to modify therapies improve clinical outcomes (CCS, 2017).

European Society of Cardiology (ESC)

The ESC states that "although there is extensive research on biomarkers in HF (e.g. ST2, galectin 3, copeptin, adrenomedullin), there is no definite evidence to recommend them for clinical practice." (Ponikowski et al., 2016)

Heart Failure Association of the European Society of Cardiology

The Heart Failure Association of the European Society of Cardiology published a position statement on Advanced Heart Failure (Crespo-Leiro et al., 2018) which states: "Post-transplant patients should undergo a pre-defined regimen of graft biopsies, titration of immunosuppressive and other therapies, rejection monitoring, assessment for infections, transplant coronary artery disease and/or cardiac allograft vasculopathy, immunosuppression side effects, and other potential complications including neoplasia, and co-morbidities that require comprehensive treatment." However, the guideline does not mention sST2 regarding prognosis of post-transplant patients (Crespo-Leiro et al., 2018).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

The Presage® ST2 Assay kit received 510(k) marketing clearance from FDA in December 2011. According to the FDA 510(k) Summary, the Presage® ST2 Assay is to be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic HF. The Presage® ST2 Assay kit is provided in a microplate configuration. The kit contains a ready-to-use 96-well microtiter plate coated with mouse monoclonal antihuman sST2 antibodies; a recombinant human sST2 standard calibrator (lyophilized); a standard diluent; an anti-ST2 biotinylated antibody reagent (mouse monoclonal antihuman sST2 antibodies) in phosphate-buffered saline; a sample diluent; a tracer concentrate and tracer diluent; a wash concentrate; a tetramethylbenzidine reagent; a stop solution; and 2 levels of controls provided in a sealed, lyophilized format (high and low control) (FDA, 2011).

Two other research products are available to assay sST2: the MBL ST2 ELISA kit (Medical and Biological Laboratories, MA) and the Human ST2/IL-1 R4 DuoSet® (R&D Systems, MN). They use different standards, different antibodies, different reagents and buffers and, thus, results are not comparable between them and the Presage® ST2 Assay. Furthermore, neither the MBL ST2 ELISA nor the Human ST2/IL-1 R4 DuoSet® assay has received FDA marketing approval. These assays are not considered in this Policy.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
83006	Growth stimulation expressed gene 2 (ST2, Interleukin 1 receptor like-1).

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes

Salivary Hormone Testing

Policy #: AHS – G2120	**Prior Policy Name & Number (as applicable):** G2120 – Salivary Hormone Testing for Menopause and Aging
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Testing of saliva has been proposed as a noninvasive method to measure free (unbound to carrier proteins) steroid hormones, including estrogen, progesterone, androgens, and cortisol, for diagnosis of hormonal imbalance and administration of individualized hormone replacement therapy (ACOG & ASRM, 2012).

Hypercortisolism can occur in several disorders, including Cushing's syndrome (pituitary hypersecretion of corticotropin/ACTH), or as a result of glucocorticoid administration resulting in obesity, hypertension, menstrual irregularity, and glucose intolerance (Lacroix, Feelders, Stratakis, & Nieman, 2015; Nieman et al., 2008; L. K. Nieman, 2019a; Quddusi, Browne, Toivola, & Hirsch, 1998).

II. Related Policies

Policy Number	Policy Title
AHS-G2161	Hormonal Testing in Adult Females
AHS-G2013	Hormonal Testing in Adult Males

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Late Night Salivary Cortisol testing **MEETS COVERAGE CRITERIA** for diagnosing Cushing's Syndrome.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

2. Salivary hormone testing for the screening, diagnosis, and/or monitoring of menopause, infertility, endometriosis, polycystic ovary disease (PCOS), premenstrual syndrome, osteoporosis, sexual dysfunction, seasonal affective disorder, depression, multiple sclerosis, sleep disorders, or diseases related to aging **DOES NOT MEET COVERAGE CRITERIA**. Salivary hormone tests include but are not limited to the following:

    a. Estrogen, including estrone (E1), estradiol (E2), and estriol (E3)
    b. Melatonin
    c. Progesterone
    d. Testosterone
    e. DHEA
    f. Cortisol

IV. Scientific Background

Testing of hormone levels in the saliva has been proposed as a noninvasive method to measure free (unbound to carrier proteins and thus active) steroid hormones (estrogen, progesterone, androgens, cortisol, etc.) for diagnosis of hormonal imbalance and administration of individualized hormone replacement therapy (ACOG & ASRM, 2012). Saliva measurements are thought to represent the concentrations of unconjugated steroid hormones as well as unconjugated steroids that have diffused freely into saliva. Conjugated steroids will often show significant decreases in concentration because their filtration process into the saliva is limited. This is what causes hormones, such as cortisol, estradiol, and testosterone to approximate concentrations well and the hormone dehydroepiandrosterone (DHEA) to represent concentrations poorly (Wood, 2009).

Salivary hormone level testing is often recommended by bioidentical hormone vendors as a means of providing personalized therapy. However, individualized testing and monitoring is only useful when a narrow therapeutic window exists for a drug or a drug class. Steroid hormones, such as estrogen and progesterone, do not meet these criteria and do not require individualized testing (ACOG & ASRM, 2012; Conaway, 2011). Furthermore, there is no evidence that hormonal levels in saliva are biologically meaningful. Saliva is an ultra- filtrate of the blood and in theory, should be amenable to testing for free concentrations of hormones; however, salivary testing does not appear to be an accurate or precise method of hormone testing (Flyckt et al., 2009; Lewis, McGill, Patton, & Elder, 2002). Studies suggest

that salivary assessments of hormone levels are inaccurate and do not correlate with levels determined from serum (Conaway, 2011), as there is large within-patient variability in salivary hormone concentrations, especially when exogenously administered hormones are given (Hardiman, Thomas, Osgood, Vlassopoulou, & Ginsburg, 1990; Klee & Heser, 2000; Lewis et al., 2002; Meulenberg, Ross, Swinkels, & Benraad, 1987; Wren et al., 2000). Salivary hormone levels often fluctuate with factors, such as circadian rhythm, and frequently do not correlate well with serum levels of hormones (Wood, 2009).

Salivary hormone measurement may be utilized for many purposes. Menopause occurs due to changing hormone levels, mainly estrogen. In general, women experience menopause at a mean age of 51 years, with most becoming menopausal between 45 and 55. Menopausal hormone therapy (MHT, estrogen alone or combined with a progestin) is used for management of menopausal symptoms and is highly effective for symptoms, such as hot flashes and vaginal atrophy. In some cases, MHT may be used for the mood lability that many women experience during the menopausal transition (Martin & Barbieri, 2020; Taylor & Manson, 2011). There are few indications for the measurement of hormone levels to evaluate success of therapy when treating a postmenopausal woman with hormones. If treatment is initiated for symptom control, therapy should be titrated to the alleviation of symptoms, not a laboratory value (ACOG & ASRM, 2012). A salivary hormone test has been developed by Genova Diagnostics, which evaluates levels of hormones in males and females during perimenopause, menopause, and andropause (male menopause) (Genova_Diagnostics, 2020).

One of the primary hormones that diffuses freely into saliva and can be well-approximated by salivary measurements is cortisol. Cortisol is a steroid hormone that is produced due to stress. Salivary flow rate does not affect cortisol concentration, and salivary cortisol correlates well with serum-free cortisol. This property can be used to identify adrenal insufficiencies and other related disorders (L. K. Nieman, 2019b). For example, the presence of Cushing's syndrome (CS) is suggested by signs of hypercortisolism, such as proximal myopathy, facial plethora, and wide purplish striae. However, none of these are pathognomonic, and many are nonspecific (such as obesity or hypertension). As a result, the diagnosis must be confirmed by biochemical tests, one of which is a salivary cortisol measurement (L. K. Nieman, 2020). The recurrence of hypercortisolemia after an initial treatment for CS seems to be predicted earlier by late night salivary cortisol (LNSC) testing compared to urinary free cortisol excretion (Fleseriu, Hamrahian, Hoffman, Kelly, & Katznelson, 2016).

Analytical Validity

Multiple proprietary tests are available for salivary hormone testing. Tests such as ZRT and UnikeyHealth ask the user to submit saliva samples and send the specimen to the proprietary lab where it can be analyzed. Labs will typically use an immunoassay-based method, such as an enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA), to assess the concentration of hormones, such as estradiol or progesterone. Others may use an automated competitive electrochemiluminescence immunoassay for LNSC measurement (Spence et al., 2018). The results are compiled into a report listing the concentrations of each hormone as well as comments on abnormal amounts. These tests are often marketed to post-menopausal women who desire to have an assessment of hormones like estrogen, progesterone, DHEA, testosterone, estriol, and cortisol (UniKey, 2018; ZRTLAB, 2018). Moreover, another proprietary test proposes that conditions such as multiple sclerosis (MS) can be assessed through irregularities in melatonin (Genova, 2019a). However, not only is

melatonin not widely measured through saliva, but there is currently no compelling data for whether administering melatonin has any utility with dealing with MS; there has been far too little published data with human subjects to draw any conclusions (Wurtman, 2017; R. Wurtman, 2019). Osteoporosis is another condition that tests may purportedly be able to screen for with saliva (Genova, 2019b). However, this test may be of limited utility as the risks of hormone therapy may outweigh the benefits (Rossouw et al., 2002).

Salivary cortisol was first measured by direct radioimmunoassay (RIA) in 1978, but more accurate cortisol immunoassays have now been developed; however, these assays are often limited due to poor specificity (El-Farhan, Rees, & Evans, 2017). Further, late at night, cortisol levels may fall below detection limits for some RIA testing methods. Liquid chromatography with tandem mass spectrometry (LC-MS-MS) has also been used for the detection of salivary cortisol. Schiffer et al. (2019) developed a novel LC-MS/MS assay to identify androgens in saliva samples with appropriate sensitivity. Prior, Li, Li, and Kellermann (2018) was able to utilize the same technique (LC-MS/MS) to accurately quantify three estrogens (estrone E1, estradiol E2, and estriol E3) in an assay with an accuracy of 98.9-112.4% and precision of (≤7.4%) as a hopeful alternative to blood samples. However, this field continues to face limitations due to poorly standardized assays and a lack of a single, validated reference range (El-Farhan et al., 2017).

Initial diagnostic tests for hypercortisolism should be highly sensitive, even if the diagnosis may be excluded later. Late night salivary cortisol (LNSC) is a first-line diagnostic test for CS as indicated by the approach outlined by the 2008 Endocrine Society (Nieman et al., 2008) and others (Hinojosa-Amaya, Varlamov, McCartney, & Fleseriu, 2019). LNSC measurements are obtained at least twice because the hypercortisolism in CS may be variable. Two measurements must be abnormal for the test to be considered abnormal; this may be especially difficult for patients with fluctuating disease. The diagnosis of CS is established when at least two different first-line tests (such as LNSC and 24-hour urinary cortisol excretion) are abnormal. Once the diagnosis is established, additional evaluation is done to identify the cause of the hypercortisolism (L. K. Nieman, 2020).

A locally modified RIA assay was developed by Nunes et al. (2009) and measured LNSC in obese patients with a current or past diagnosis of CS. The assay was able to diagnose a recurrence of CS with a sensitivity of 90% and a specificity of 91.8%; it was also reported that "A threshold of 12 nmol/liter yielded 100% sensitivity and specificity in overt Cushing's syndrome" (Nunes et al., 2009).

Clinical Validity and Utility

A study by Lewis et al. (2002) focusing on salivary progesterone measurements found major variation when a progesterone cream was applied to several post-menopausal women. Salivary measurements were collected at 0, 1, 3, 4, 7, and 8 weeks. The average baseline for the 20 mg/g cream group was found to be 0.25 ± 0.12 nmol/L, but the measurement at 1 week was 82.11 ± 104.52 nmol/L (Lewis et al., 2002); similar enormous variations were found at 3 and 7 weeks, as well as the 40 mg/gm cream group. In contrast, the placebo group's baseline was 0.43±0.21 and 0.38±0.20 in week 8 (Lewis et al., 2002). The finding with inconsistent salivary progesterone levels was even found among premenopausal women obtaining *in vitro* fertilization (IVF); on the other hand, salivary estradiol was found to be

correlative to serum-based assessment, and could be a less invasive alternative to blood draws for ovarian stimulation during IVF cycles (Sakkas et al., 2020).

LNSC measurements were found to be concordant with the 24-hour urine test, with 97% concordance at ≥4 nmol/L and 69% concordance at ≥10 nmol/L. However, the tests were stated to be "equivalent" at the more sensitive cutoff of 4 nmol/L. The authors concluded that due to the concordance of the salivary test with the urine test, the salivary test should replace the urinary test as the frontline test for Cushing's syndrome (Doi, Clark, & Russell, 2013). Another study found LNSC to be 100% sensitive and 98% specific at a cut-off of 2.4 nmol/L (Antonelli, Ceccato, Artusi, Marinova, & Plebani, 2015). Both cortisol and its metabolite cortisone were tested as cortisone is a significant source of interference in certain immunoassays. The variation between and within runs were both under 10%, the method was linear up to 55.4 nmol/L for cortisol, and the lower of limit of quantification was 0.51 nmol/L for cortisol (Antonelli et al., 2015).

A study measured the utility of salivary testosterone and cortisol concentrations in 71 junior athletes (26 females and 45 males) in response to stress. The researchers compared results of salivary samples to capillary blood samples taken at the same time; while blood samples showed an increase in both testosterone and cortisol concentrations in both sexes, salivary samples showed no change in testosterone or cortisol levels (Crewther, Obminski, Orysiak, & Al-Dujaili, 2018). This may suggest that salivary hormone testing in these populations is not as efficient as other methods.

Valassi et al. (2017) analyzed diagnostic data from 1341 CS patients in the European Registry on Cushing's syndrome (ERCUSYN) and noted that of the three main first-line CS diagnostic tests, the urinary free cortisol test was performed in 78% of patients as a first-line testing method, overnight 1 mg dexamethasone suppression test was performed in 60% of patients, and LNSC was performed in only 25% of patients. This shows that LNSC may not be used as frequently as other testing methods for a first-line diagnosis of CS.

Salivary testing for cortisol could also prove useful in occupational settings as a parameter for stress. Oldenburg and Jensen (2019) conducted a study on merchant ship crew, and found that after adjustment, average salivary cortisol level was positively associated with "acute shipboard stressors, namely the average current working time (p=.050) and the average number of terminals that had been served during the last 7 days (p=0.008)." This laboratory data is essential in all fields wherein professionals experience high levels of stress, so that measures can be taken to create a positive working environment.

V. Guidelines and Recommendations

American Association of Clinical Endocrinologists (AACE) (Goodman, Cobin, Ginzburg, Katz, & Woode, 2011)

The AACE has noted salivary hormone level testing as recommended by certain proponents to provide individualized therapy. However, these methods are not FDA or CLIA approved, and factors such as hydration and circadian rhythm may influence the concentration of hormones within a subject. Standardization is difficult, and even though standardized blood tests do exist, it is of limited clinical

utility because measuring hormone levels in postmenopausal women has no predictive value on what the normal levels should be. A salivary measurement cannot be used to correct the levels of sex hormones (Goodman et al., 2011).

American College of Obstetricians and Gynecologists (ACOG) and the American Society of Reproductive Medicine Practice Committee (ASRM) [(ACOG & ASRM, 2012) reaffirmed 2020]

ACOG and ASRM released joint guidelines on compounded hormone therapy that stated salivary hormone testing had no evidence to support its biological utility and that testing the hormone levels were neither accurate nor precise. The guidelines stated that salivary hormone testing had large intra-patient variability depending on factors such as diet and that saliva did not provide a reasonable representation of serum hormone levels. Saliva may be contaminated with other cell types, contains lower concentration of hormones than serum, and impossible to reliably test for a representative result. The guidelines concluded that evidence is inadequate to support an individualized hormone therapy based on salivary, serum, or urine testing (ACOG & ASRM, 2012).

Finally, the guideline wrote that "there is no evidence that hormonal levels in saliva are biologically meaningful." (ACOG & ASRM, 2012)

North American Menopausal Society (NAMS, 2012, 2017)

The NAMS addressed salivary hormone testing with regards to MHT, stating that salivary hormone testing is "inaccurate and unreliable." The NAMS further notes that the levels in serum, saliva, and tissue are "markedly different" and alludes to the FDA's statement that there is "no scientific basis for using saliva testing to adjust hormone levels" (NAMS, 2012).

The NAMS also addressed salivary hormone testing in the context of compounded HT (hormone therapy), which would include estradiol, estrone, and micronized progesterone (MP), but corroborates that salivary testing for HT is considered "unreliable because of differences in hormone pharmacokinetics and absorption, diurnal variation, and inter-individual and intraindividual variability" (NAMS, 2017).

Endocrine Society (ES) (Nieman, 2015; Santoro et al., 2016)

The ES states that "salivary hormone assays are not standardized, do not have independent quality control programs, and lack an accepted reference range." The Society further mentions that there is no scientific evidence that a correlation exists between symptoms and salivary hormones. Assessment or monitoring of hormone therapy lacks evidence, and the American College of Obstetricians and Gynecologists, the North American Menopausal Society, and the Endocrine Society all recommend against salivary hormone testing (Santoro et al., 2016).

The ES also recommends a test of at least two LNSC measurements for diagnosis of Cushing's Syndrome. If a patient has eucortisolism after a transsphenoidal selective adenomectomy (TSS), a measurement of late-night salivary or serum cortisol is recommended (Nieman, 2015; Nieman et al., 2008).

VI. State and Federal Regulations (as applicable)

Salivary hormones may be measured by multiple tests. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82530	Cortisol free
82533	Cortisol; total
82626	Dehydroepiandrosterone (dhea)
82627	Dehydroepiandrosterone
82670	Estradiol; total
82671	Estrogens; fractionated
82672	Estrogens; total
82677	Estriol
82679	Estrone
82681	Estradiol; free, direct measurement (e.g., equilibrium dialysis)
84144	Progesterone
84402	Testosterone; free
84403	Testosterone; total
84410	Testosterone; bioavailable, direct measurement (e.g., differential precipitation)
S3650	Saliva test, hormone level; during menopause

Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes

Serum Biomarker Testing for Multiple Sclerosis and Related Neurologic Diseases

Policy #: AHS – G2123	**Prior Policy Name & Number (as applicable):** AHS – G2123 – Serum Biomarker Tests for Multiple Sclerosis
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Multiple sclerosis (MS) is the most common immune-mediated inflammatory demyelinating disease of the central nervous system (CNS) defined by multifocal areas of demyelination with loss of oligodendrocytes and astroglial scarring. The most common presenting symptoms are sensory disturbances, followed by weakness and visual disturbances, but the disease has a highly variable pace and many atypical forms (Olek, 2019a). Besides MS, acute CNS demyelination also occurs in acute disseminated encephalomyelitis (ADEM), optic neuritis, transverse myelitis, and neuromyelitis optica (Lotze, 2019).

Neuromyelitis optica and neuromyelitis optica spectrum disorders (NMOSD) are inflammatory disorders of the CNS characterized by severe, immune-mediated demyelination and axonal damage predominantly targeting the optic nerves and spinal cord. This set of disorders was previously considered a subset of MS, but is now recognized as its own clinical entity with its own unique immunologic features (Glisson, 2019).

II. Related Policies

Policy Number	Policy Title
	Not applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1) For the diagnosis of multiple sclerosis (MS), cerebrospinal fluid (CSF) and serum oligoclonal band analysis **MEETS COVERAGE CRITERIA** in any of the following situations
   a) For individuals with atypical clinical, laboratory, or imaging features.
   b) For individuals with an atypical, clinically isolated syndrome, including, but not limited to, primary progressive multiple sclerosis or relapsing-remitting course.
   c) For individuals belonging to a population in which MS is less common, including, but not limited to, children, older individuals, or non-Caucasians.
   d) For individuals with insufficient clinical or imaging evidence for diagnosis.

2) In cases of suspected neuromyelitis optica spectrum disorders (NMOSD) or myelin oligodendrocyte glycoprotein-immunoglobulin G (MOG-IgG)-associated encephalomyelitis (MOG-EM), serum indirect fluorescence assay or fluorescence-activated cell sorting (FACS) assay of aquaporin-4-IgG (AQP4-IgG) and MOG-IgG **MEET COVERAGE CRITERIA** when all of the following conditions are met:
   a) The individual has monophasic or relapsing acute optic neuritis, myelitis, brainstem encephalitis, encephalitis, or any combination thereof;
   b) The individuals have radiological or electrophysiological findings compatible with CNS demyelination;
   c) The individual has at least one of the following:
      i) Belongs to a higher risk population—African American, Latin American, Asian, or pediatric.
      ii) Has an abnormal MRI depicting extensive optic nerve lesion, extensive spinal cord lesion or atrophy, or large confluent T2 brain lesions.
      iii) Has prominent papilledema/papillitis/optic disc swelling during acute optic neuritis.
      iv) Has neutrophilic CSF pleocytosis.
      v) Has a histopathology finding of primary demyelination with intralesional complement and IgG deposits or has a previous diagnosis of "pattern II MS".

vi) Has simultaneous bilateral acute optic neuritis.

vii) Has a severe visual deficit or blindness in one or both eyes during or after acute optic neuritis.

viii) Has severe or frequent episodes of acute myelitis or brainstem encephalitis.

ix) Has permanent sphincter and/or erectile disorder after myelitis.

x) Has a previous diagnosis of acute disseminated encephalomyelitis (ADEM).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3) In all other situations, serum biomarker tests for multiple sclerosis **DO NOT MEET COVERAGE CRITERIA**.

4) ELISA, Western blot, immunohistochemistry, or any other serum assays to test for NMOSD or MOG-EM **DO NOT MEET COVERAGE CRITERIA**.

5) For the diagnosis of MS, NMOSD, or MOG-EM, all other cerebrospinal fluid (CSF) biomarker tests, including AQP4-IgG or MOG-IgG, **DO NOT MEET COVERAGE CRITERIA.**

IV. Scientific Background

In the United States, the 2010 estimated prevalence of multiple sclerosis (MS) is 309.2 per 100,000 individuals, totaling 727,344 persons with MS (Wallin et al., 2019). The mean age of MS onset is 28 to 31 years of age with clinical disease usually becoming apparent between the ages of 15 to 45 years, though in rare instances, onset has been noted as early as the first years of life or as late as the seventh decade (Goodin, 2014). Prevalence of MS is highest in the 55- to 65- year age group (Wallin et al., 2019). In most, but not all, cases, a patient presents with a clinically isolated syndrome (CIS) as the first single clinical event. This CIS preludes a clinically definite MS (Lublin et al., 2014). The pattern and course of MS is then further categorized into several clinical subtypes (Lublin & Reingold, 1996; Lublin et al., 2014): Relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS). RRMS is the most common type of disease course (85 to 90 percent of cases at onset (Weinshenker, 1994)) and is characterized by clearly defined relapses with full recovery, or with sequelae and residual deficit upon recovery. The transition from RRMS to SPMS usually occurs 10 to 20 years after disease onset (Eriksson et al., 2003). SPMS is characterized by an initial RRMS disease course followed by gradual worsening with or without occasional relapses, minor remissions, and plateaus. PPMS is characterized by progressive accumulation of disability from disease onset with occasional plateaus, temporary minor improvements, or acute relapses still consistent with the definition. A diagnosis of PPMS is made exclusively on patient history: there are no imaging or exam findings that distinguish PPMS from RRMS. PPMS represents about 10 percent of MS cases at disease onset (Koch et al., 2009; Olek, 2022a). Worsening of disability due to MS is highly variable. The impact of MS varies according to several measures, including severity of signs and symptoms, frequency of relapses, rate of worsening, and residual disability. Worsening of disability over time is a critical issue for MS patients (Olek, 2022a). Current treatments can delay the progression of the disease. However, this delay is only achievable if treatment starts at the beginning of the disease. Thus, it is essential that

a proper diagnosis is made as early as possible, allowing for early treatment and as much delay as possible in symptom progression (Sapko et al., 2020).

Multiple sclerosis is primarily diagnosed clinically. The core requirement for the diagnosis is the demonstration of central nervous system lesion dissemination in time and space, based upon either clinical findings alone or a combination of clinical and MRI findings. The history and physical examination are most important for diagnostic purposes. MRI is the test of choice to support the clinical diagnosis of MS (Filippi & Rocca, 2011). The McDonald diagnostic criteria include specific MRI criteria for the demonstration of lesions dissemination in time and space; however, the McDonald criteria are not intended for distinguishing MS from other neurologic conditions (Brownlee et al., 2017). The sensitivity and specificity of MRI for the diagnosis of MS varies widely in different studies. This variation is probably due to differences among the studies in MRI criteria and patient populations (Offenbacher et al., 1993; Schaffler et al., 2011). Using the 2010 McDonald criteria, the sensitivity and specificity were approximately 53 and 87 percent, respectively (Rovira et al., 2009). In the first studies applying the 2017 criteria (Hyun et al., 2018), the sensitivity is higher (83.6%), but the specificity is lower (85%).

Qualitative assessment of cerebrospinal fluid (CSF) for oligoclonal IgG bands (OCBs) using isoelectric focusing can be an important diagnostic tool when determining a diagnosis of MS. Elevation of the CSF immunoglobulin level relative to other protein components is a common finding in patients with MS and suggests intrathecal synthesis. The immunoglobulin increase is predominantly IgG, although the synthesis of IgM and IgA is also increased (Olek, 2022a). A positive finding is defined by "finding of either oligoclonal bands different from any such bands in serum, or by an increased IgG index" and can be measured by features such as percentage of total protein or total albumin. Up to 95% of clinically definite MS cases will have these oligoclonal bands (Olek, 2022b).

The 2017 McDonald criteria allows for the presence of CSF oligoclonal bands to substitute for the diagnostic requirement of fulfilling dissemination in time. However, Thompson notes that "currently, no laboratory test in isolation confirms the diagnosis of multiple sclerosis" (Thompson et al., 2018). Luzzio (2019) et al also note that in a review of four guidelines from the Consortium of Multiple Sclerosis Centers, the European Academy of Neurology, and the Magnetic Resonance Imaging in MS Network, MRI is the "imaging procedure of choice for confirming MS and monitoring disease progression in the brain and spinal cord" (Luzzio, 2019).

Neuromyelitis optica spectrum disorders (NMOSD, also known as Devic disease or neuromyelitis optica, NMO) are a range of conditions that are characterized by symptoms similar to MS; namely demyelination and axonal damage to structures of the central nervous system, such as the spinal cord. Previously, NMOSD were considered a subset of MS; however, now NMOSD and NMO are recognized as having distinct features, specifically the presence of a NMOSD/NMO-specific antibody that binds aquaporin-4 (AQP4), setting these apart from relapsing-remitting MS. AQP4 is a water channel protein primarily located in the spinal cord gray matter. NMO-IgG (or anti-AQP4) is involved in the pathogenesis of NMOSD/NMO. This antibody selectively binds AQP4, differing from MS in that the loss of AQP4 expression is unrelated to the stage of demyelination. The presence of this antibody is incorporated into the current diagnostic criteria for NMOSD and can differentiate MS cases from NMOSD cases (Glisson, 2022).

Clinical Utility and Validity

There is a strong unmet clinical need for objective body fluid biomarkers to assist early diagnosis and estimate long-term prognosis, monitor treatment response, and predict potential adverse effects in MS. Currently, no biomarkers of MS have been validated; however, many are under consideration: microRNA (miRNA), messenger RNA (mRNA), lipids, autoantibodies, metabolites, and proteins all have been reported to have potential as possible biomarkers (Comabella & Montalban, 2014; Comabella et al., 2016; El Ayoubi & Khoury, 2017; Lim et al., 2017; Raphael et al., 2015; Teunissen et al., 2015).

Fryer et al. (2014) compared three assays for measuring aquaporin-4 IgG: ELISA, fixed cell-based fluorescence (CBA), and live cell-based fluorescence (FACS, M1 and M23 versions). Four groups of patients were measured with these assays. In Group 1 (n = 388), FACS was optimal, with the highest area under the curve. In Group 2, FACS identified the highest percentage of neuromyelitis optica spectrum disorders, identifying 23 (M1) and 24 (M23) of 30 patients. In Group 3, all four assays identified true negatives at an approximate 85% success rate (5 of 31 positives). In Group 4, all four assays identified true positives in 40 of 41 samples. The authors noted that "aquaporin-4-transfected CBAs, particularly M1-FACS, perform optimally in aiding NMOSD serologic diagnosis" (Fryer et al., 2014).

Jitprapaikulsan et al. (2018) evaluated the prognostic value of aquaporin-4 IgG and myelin oligodendrocyte glycoprotein IgG (MOG) in patients with recurrent optic neuritis (rON). The study included 246 and autoantibodies were detected in 32% of these patients (aquaporin-4 in 19%, MOG in 13%). 186 patients had rON only and 60 patients had "additional inflammatory demyelinating attacks" (rON plus). Of the 186 rON only patients, 27 were positive for MOG, 24 were positive for aquaporin-4, and 110 were negative for both. In the rON plus group, 23 were positive for aquaporin-4, 4 were positive for MOG, and 11 were negative for both. The authors noted that 5 years after optic neuritis onset, 59% of aquaporin-4 positive patients and 12% of MOG positive patients were estimated to have "severe visual loss". The authors concluded that "aquaporin-4 IgG seropositivity predicts a worse visual outcome than MOG IgG1 seropositivity, double seronegativity, or MS diagnosis. Myelin oligodendrocyte glycoprotein IgG1 is associated with a greater relapse rate but better visual outcomes" (Jitprapaikulsan et al., 2018).

Sotirchos et al. (2019) compared 31 healthy controls with individuals with one of three types of optic neuritis (ON): 48 individuals with aquaporin-4 IgG-associated ON (AQP4-ON), 16 individuals with myelin oligodendrocyte glycoprotein-IgG-associated ON (MOG-ON), and 40 individuals with MS-associated ON (MS-ON). The authors note, "AQP4-ON eyes exhibited worse high-contrast letter acuity (HCLA) compared to MOG-ON (-22.3 ± 3.9 letters; $p < 0.001$) and MS-ON eyes (-21.7 ± 4.0 letters; $p < 0.001$). Macular ganglion cell + inner plexiform layer (GCIPL) thickness was lower, as compared to MS-ON, in AQP4-ON (-9.1 ± 2.0 µm; $p < 0.001$) and MOG-ON (-7.6 ± 2.2 µm; $p = 0.001$) eyes. Lower GCIPL thickness was associated with worse HCLA in AQP4-ON (-16.5 ± 1.5 letters per 10 µm decrease; $p < 0.001$) and MS-ON eyes (-8.5 ± 2.3 letters per 10 µm decrease; $p < 0.001$), but not in MOG-ON eyes (-5.2 ± 3.8 letters per 10 µm decrease; $p = 0.17$), and these relationships differed between the AQP4-ON and other ON groups ($p < 0.01$ for interaction)." These data indicate that AQP4-IgG seropositivity suggests worse visual outcomes than those occurring after MOG-ON or even MS-ON (Sotirchos et al., 2019).

Cantó et al. (2019) evaluated neurofilament light chain's (NfL) ability to "serve as a reliable biomarker of disease worsening for patients with multiple sclerosis (MS)." The study included 607 patients with MS; patients were assessed over a period of 12 years. Serum NfL was measured, and disability progression

was the primary clinical outcome (defined as "clinically significant worsening on the Expanded Disability Status Scale (EDSS) score and brain fraction atrophy"). Baseline measurements of NfL showed significant association with EDSS score, MS subtype, and treatment status. Worsening EDSS scores and changes of NfL levels over time were found to be correlated. The baseline NfL measurement was also found to be associated with approximately 11.6% of brain fraction atrophy over 10 years, increasing to 18% after multivariable analysis. Furthermore, active treatment was associated with declining levels of NfL, with "high-potency treatments" associated with the greatest decrease out of all of the treatments assessed. Overall, the authors concluded that they had confirmed a significant association of serum NfL with clinical outcomes of MS. However, they also acknowledged that "further prospective studies are necessary to assess the assay's utility for decision-making in individual patients" (Cantó et al., 2019).

Gil-Perotin et al. (2019) evaluated the combined biomarker profile of NfL and chitinase3-like1 (CHI3L1) and its ability to provide prognostic information for patients with MS. 157 MS patients were included, with 99 RRMS patients, 35 SPMS patients, and 23 PPMS patients. Disease activity was defined by "clinical relapse and/or gadolinium-enhanced lesions (GEL) in MRI within 90 days from CSF collection." Levels of both biomarkers were found to be higher in MS patients compared to non-MS patients. Elevated NfL was associated with clinical relapse and GEL in RRMS and SPMS patients and high CHI3L1 levels were characteristic of progressive disease. The authors also found the combined profile useful for differentiating between MS subtypes, with high NfL and low CHI3L1 often indicating a RRMS stage. They found that elevation of both biomarkers indicates disease progression. Overall, the authors concluded these biomarkers were useful for disease activity and progression and that the biomarker profile can discriminate between MS subtypes (Gil-Perotin et al., 2019).

Martin et al. (2019) performed a meta-analysis to evaluate the CSF levels of NfL to determine "whether, and to what degree, CSF NfL levels differentiate MS from controls, or the subtypes or stages of MS from each other". The authors identified 14 articles for inclusion in their meta-analysis. NfL levels were higher in MS patients (746) than controls (435) (mean of 1965.8 ng/L in MS patients compared to 578.3 ng/L in healthy controls). Mean NfL levels were found to be higher in 176 patients with relapsing disease (mean = 2124.8ng/L) compared to 92 patients with progressive disease (mean = 1121.4ng/L). The authors also found that patients with relapsing disease (138 in this cohort) had approximately double the levels of CSF NfL compared to patients in remission (268), with an average of 3080.6ng/L in the relapsing cohort compared to 1541.7ng/L in the remission cohort. Overall, the authors concluded that CSF NfL correlates with MS activity throughout the course of disease, that relapse was strongly associated with elevated CSF NfL levels, and that CSF NfL may be useful as a measure of activity (Martin et al., 2019).

Simonsen et al. (2020) performed a retrospective study investigating if analysis of IgG index could safely predict oligoclonal band (OCB) findings. 1295 MS patients were included, with 93.8% of them positive for OCBs. Of 842 MS patients with known IgG status and known OCB status, 93.3% were oligoclonal band positive and 76.7% were found to have an elevated IgG profile. The authors found the positive predictive value of elevated IgG based on positive OCBs to be 99.4%, and the negative predictive value of normal IgG based on negative OCBs to be 26.5%. The authors concluded that an IgG index of >0.7 has a positive predictive value of >99% for OCBs (Simonsen et al., 2020).

Benkert et al. (2022) conducted a retrospective modelling and validation study aiming to assess the ability of serum neurofilament light chain (sNfL) to identify people at risk of future MS. The authors used a reference database to determine reference values of sNfL corrected for age and body mass index

(BMI). The study included a control group (no history of CNS disease) and MS patients. In the control group, sNfL concentrations increased exponentially with age; the rate of increase rose after the age of 50. In MS patients, "sNfL percentiles and Z scores indicated a gradually increased risk for future acute (eg, relapse and lesion formation) and chronic (disability worsening) disease activity." The authors collected data before and after MS treatment and found that sNfL Z score values decreased to the level of the control group with monoclonal antibodies, and, to a lesser extent, with oral therapies. sNfL Z scores did not decrease with platform compounds such as interferons and glatiramer acetate. The authors conclude that "use of sNfL percentiles and Z scores allows for identification of individual people with multiple sclerosis at risk for a detrimental disease course and suboptimal therapy response beyond clinical and MRI measures, specifically in people with disease activity-free status" (Benkert et al., 2022).

V. Guidelines and Recommendations

International Advisory Committee on Clinical Trials in Multiple Sclerosis

In 2014, the International Advisory Committee on Clinical Trials in Multiple Sclerosis, jointly sponsored by the U.S. National Multiple Sclerosis Society, the European Committee for Treatment and Research in Multiple Sclerosis, and the MS Phenotype Group, re-examined MS phenotypes, exploring clinical, imaging, and biomarker advances through working groups and literature searches. The committee concluded that "To date, there are no clear clinical, imaging, immunologic or pathologic criteria to determine the transition point when RRMS [relapse-remitting MS] converts to SPMS [secondary progressive MS]; the transition is usually gradual. This has limited our ability to study the imaging and biomarker characteristics that may distinguish this course" (Lublin et al., 2014). In 2020, the committee updated this policy for clarity, summarizing with "the committee urges clinicians, investigators, and regulators to consistently and fully use the 2013 phenotype characterizations by (1) using the full definition of activity, that is, the occurrence of a relapse or new activity on an MRI scan (a gadolinium-enhancing lesion or a new/unequivocally enlarging T2 lesion); (2) framing activity and progression in time; and (3) using the terms worsening and progressing or disease progression more precisely when describing MS course"(Lublin et al., 2020).

The International Panel on Diagnosis of Multiple Sclerosis

The Panel reviewed the 2010 McDonald criteria and recommended: "In a patient with a typical clinically isolated syndrome and fulfilment of clinical or MRI criteria for dissemination in space and no better explanation for the clinical presentation, demonstration of CSF-specific oligoclonal bands in the absence of other CSF findings atypical of multiple sclerosis allows a diagnosis of this disease to be made." The Panel goes on to state that "CSF oligoclonal bands are an independent predictor of the risk of a second attack when controlling for demographic, clinical, treatment, and MRI variables" and that in the absence of atypical CSF findings, demonstration of these CSF OCBs can allow for a diagnosis of MS to be made. The Panel remarks that inclusion of this CSF criterion can substitute for the traditional "dissemination in time" criterion, but that no laboratory test in isolation can confirm an MS diagnosis (Thompson et al., 2018).

Cerebrospinal fluid examination is "strongly recommended" in some circumstances for MS diagnosis, and the Panel remarks that the threshold for additional testing should be low. Those circumstances are as follows:

- "when clinical and brain MRI evidence supporting a diagnosis of multiple sclerosis is insufficient, particularly if initiation of long-term disease-modifying therapies are being considered"
- "when there is a presentation other than a typical clinically isolated syndrome, including patients with a progressive course at onset (primary progressive multiple sclerosis)"
- "when there are clinical, imaging, or laboratory features atypical of MS"
- "in populations in which diagnosing MS is less common (for example, children, older individuals, or non-Caucasians)."

The Panel does emphasize that it is essential for CSF to be paired with another serum sample when analyzed to demonstrate that the OCBs are unique to the CSF (Thompson et al., 2018).

The treatments for these similar conditions (MS and NMOSD) differ, as some MS treatments (interferon beta, fingolimod, and natalizumab) can exacerbate NMOSDs. Therefore, the Panel recommended that "NMOSDs should be considered in any patient being evaluated for multiple sclerosis". The Panel notes that aquaporin-4 serological testing "generally differentiates" NMOSD from MS (Thompson et al., 2018). Serological testing for AQP4 and for MOG should be done in all patients with features suggesting NMOSDs (severe brainstem involvement, bilateral optic neuritis, longitudinally extensive spinal cord lesions, large cerebral lesions, or a normal brain MRI or findings not fulfilling dissemination in space [DIS]), and considered in groups at higher risk of NMOSDs (African American, Asian, Latin American, and pediatric populations) (Thompson et al., 2018).

International Panel on MOG Encephalomyelitis

Human myelin oligodendrocyte glycoprotein (MOG-IgG)-associated encephalomyelitis (MOG-EM) is considered a unique disease from MS and other NMOSD, but MOG-EM has often been misdiagnosed as MS in the past. In 2018, an international panel released their recommendations concerning diagnosis and antibody testing. They state their purpose with the following: "To lessen the hazard of overdiagnosing MOG-EM, which may lead to inappropriate treatment, more selective criteria for MOG-IgG testing are urgently needed. In this paper, we propose indications for MOG-IgG testing based on expert consensus. In addition, we give a list of conditions atypical for MOG-EM ("red flags") that should prompt physicians to challenge a positive MOG-IgG test result. Finally, we provide recommendations regarding assay methodology, specimen sampling and data interpretation" (Jarius et al., 2018).

They list the following recommendations:

- Assay: Indirect fluorescence assays, including fluorescence-activated cell sorting (FACS) that targets full-length human MOG (IgG-specific), are the gold standards. The use of either IgM or IgA antibodies are less specific and can result in both false-negative results due to high-affinity IgG displacing IgM and false-positive results due to cross-reactivity with rheumatoid factors.
- Immunohistochemistry is NOT recommended because it is "less sensitive than cell-based assays, limited data available on specificity, [and] sensitivity depends on tissue donor species."
- Peptide-based ELISA and Western blot are NOT recommended because they are "insufficiently specific, obsolete."

Serum Biomarker Testing for Multiple Sclerosis and Related Neurologic Diseases, continued

- Biomaterial: Serum is the recommended specimen of choice. CSF is "not usually required" because "MOG-IgG is produced mostly extrathecally, resulting in lower CSF than serum titers."
- Timing of testing: Serum concentration of MOG-IgG is highest during an acute attack and/or while not receiving immunosuppressive treatment. MOG-IgG concentration may decrease during remission. "If MOG-IgG test is negative but MOG-EM is still suspected, re-testing during acute attacks, during treatment-free intervals, or 1-3 months after plasma exchange (or IVIG [intravenous immunoglobulin treatment]) is recommended."
- "Given the very low pre-test probability, we recommend against general MOG-IgG testing in patients with a progressive disease course."
- "In practice, many patients diagnosed with AQP4-IgG-negative NMOSD according to the IPND 2015 criteria will meet also the criteria for MOG-IgG testing…and should thus be tested. However, MOG-IgG testing should not be restricted to patients with AQP4-IgG-negative NMOSD" (Jarius et al., 2018).

The table below outlines the recommendation on the criteria required for testing:

Table 1 Recommended indications for MOG-IgG testing in patients presenting with acute CNS demyelination of putative autoimmune etiology

1. Monophasic or relapsing acute optic neuritis, myelitis, brainstem encephalitis, encephalitis, or any combination thereof,
and
2. radiological or, only in patients with a history of optic neuritis, electrophysiological (VEP) findings compatible with CNS demyelination,
and
3. at least one of the following findings:

MRI
- a. Longitudinally extensive spinal cord lesion (≥3 VS, contiguous) on MRI (so-called LETM)[a,b]
- b. Longitudinally extensive spinal cord atrophy (≥3 VS, contiguous) on MRI in patients with a history compatible with acute myelitis[a]
- c. Conus medullaris lesions, especially if present at onset[c]
- d. Longitudinally extensive optic nerve lesion (e.g., >1/2 of the length of the pre-chiasmal optic nerve, T2 or T1/Gd)[d]
- e. Perioptic Gd enhancement during acute ON[e]
- f. Normal supratentorial MRI in patients with acute ON, myelitis and/or brainstem encephalitis
- g. Brain MRI abnormal but no lesion adjacent to a lateral ventricle that is ovoid/round or associated with an inferior temporal lobe lesion and no Dawson's finger-type or juxtacortical U fiber lesion (Matthews-Jurynczyk criteria[f])
- h. Large, confluent T2 brain lesions suggestive of ADEM

Fundoscopy
- i. Prominent papilledema/papillitis/optic disc swelling during acute ON

CSF
- j. Neutrophilic CSF pleocytosis[g] or CSF WCC > 50/µl[h]
- k. No CSF-restricted OCB as detected by IEF at first or any follow-up examination[i] (applies to continental European patients only)

Histopathology
- l. Primary demyelination with intralesional complement and IgG deposits
- m. Previous diagnosis of "pattern II MS"[j]

Clinical findings
- n. Simultaneous bilateral acute ON
- o. Unusually high ON frequency or disease mainly characterized by recurrent ON
- p. Particularly severe visual deficit/blindness in one or both eyes during or after acute ON
- q. Particularly severe or frequent episodes of acute myelitis or brainstem encephalitis
- r. Permanent sphincter and/or erectile disorder after myelitis
- s. Patients diagnosed with "ADEM", "recurrent ADEM", "multiphasic ADEM" or "ADEM-ON"
- t. Acute respiratory insufficiency, disturbance of consciousness, behavioral changes, or epileptic seizures (radiological signs of demyelination required)
- u. Disease started within 4 days to ~4 weeks after vaccination
- v. Otherwise unexplained intractable nausea and vomiting or intractable hiccups (compatible with area postrema syndrome)[a]
- w. Co-existing teratoma or NMDAR encephalitis (low evidence[k])

Treatment response
- x. Frequent flare-ups after IVMP, or steroid-dependent symptoms[l] (including CRION)
- y. Clear increase in relapse rate following treatment with IFN-beta or natalizumab in patients diagnosed with MS (low evidence)

International Panel on NMOSD

The International Panel on NMOSD recommends "testing with cell-based serum assays (microscopy or flow cytometry-based detection) whenever possible because they optimize autoantibody detection (mean sensitivity 76.7% in a pooled analysis; 0.1% false-positive rate in a MS clinic cohort)." They state that ELISA and indirect immunofluorescence assays have lower sensitivity and "strongly" recommend "interpretative caution if such assays are used and when low-titer positive ELISA results are detected in individuals who present with NMOSD clinical symptoms less commonly associated with AQP4-IgG (e.g., presentations other than recurrent optic neuritis, myelitis with LETM, or area postrema syndrome) or in situations where clinical evidence suggests a viable alternate diagnosis. Confirmatory testing is recommended, ideally using 1 or more different AQP4-IgG assay techniques. Cell-based assay has the best current sensitivity and specificity and samples may need to be referred to a specialized laboratory." The table below outlines the NMOSD diagnostic criteria for adult patients (Wingerchuk et al., 2015).

Table 1 NMOSD diagnostic criteria for adult patients

Diagnostic criteria for NMOSD with AQP4-IgG

1. At least 1 core clinical characteristic
2. Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended)
3. Exclusion of alternative diagnoses[a]

Diagnostic criteria for NMOSD without AQP4-IgG or NMOSD with unknown AQP4-IgG status

1. At least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements:
   a. At least 1 core clinical characteristic must be optic neuritis, acute myelitis with LETM, or area postrema syndrome
   b. Dissemination in space (2 or more different core clinical characteristics)
   c. Fulfillment of additional MRI requirements, as applicable
2. Negative tests for AQP4-IgG using best available detection method, or testing unavailable
3. Exclusion of alternative diagnoses[a]

Core clinical characteristics

1. Optic neuritis
2. Acute myelitis
3. Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting
4. Acute brainstem syndrome
5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions (figure 3)
6. Symptomatic cerebral syndrome with NMOSD-typical brain lesions (figure 3)

National Institute for Health and Care Excellence (NICE)

The 2022 NICE guidelines on MS in adults recommends diagnosing MS using "combination of history, examination, MRI and laboratory findings, and by following the 2017 revised McDonald criteria" and

notes that this should include "looking for cerebrospinal fluid-specific oligoclonal bands if there is no clinical or radiological evidence of lesions developing at different times" (NICE, 2022).

VI. Applicable State and Federal Regulations

In 2016, the FDA approved the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay. The indication for use is as follows: "The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay is for the semi-quantitative determination of autoantibodies to Aquaporin-4 in human serum. The KRONUS Aquaporin 4 Autoantibody (AQP4Ab) ELISA Assay may be useful as an aid in the diagnosis of Neuromyelitis Optica (NMO) and Neuromyelitis Optica Spectrum Disorders (NMOSD). The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay is not to be used alone and is to be used in conjunction with other clinical, laboratory, and radiological (e.g. MRI) findings" (FDA, 2016).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83916	Oligoclonal immune (oligoclonal bands)
84182	Protein; Western Blot, with interpretation and report, blood or other body fluid, immunological probe for band identification, each
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Serum Testing for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease

Policy #: AHS – G2110	Prior Policy Name & Number (as applicable): • AHS – G2110 – Multianalyte Assays with Algorithmic Analysis for the Evaluation and Monitoring of Patients with Chronic Liver Disease • AHS – G2110 – Serum Marker Panels for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease
Implementation Date: 9/15/21	**Date of Last Revision:** 11/12/21, 2/13/23 (See Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Chronic liver disease (CLD) refers to a wide range of liver pathologies that include inflammation (chronic hepatitis), liver cirrhosis, and hepatocellular carcinoma.

Hepatic fibrosis is associated with a cycle of extracellular matrix deposition and degradation. Biomarkers of extracellular matrix turnover are used to directly assess fibrosis and, theoretically, to monitor progression or regression (Valva, Rios, De Matteo, & Preciado, 2016). These markers include several glycoproteins, members of the collagen family, collagenases and their inhibitors, and a number of cytokines involved in the fibrogenic process (Valva et al., 2016). The markers may be utilized individually, as well as in panel combinations (Parikh et al., 2017).

II. Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis C
AHS-G2124	Serum Tumor Markers for Malignancies
AHS-G2173	Gamma-glutamyl Transferase

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

The preferred test for assessing fibrosis is FibroScan.

1) For individuals with hepatitis C, hepatitis B, nonalcoholic fatty liver disease (NAFLD) (including nonalcoholic steatohepatitis (NASH)), **or** alcoholic hepatitis, the use of the following multianalyte assays with algorithmic analysis to distinguish hepatic cirrhosis from non-cirrhosis **MEETS COVERAGE CRITERIA**:
   a) ELF™(ELFTM).
   b) FibroTest ®.
   c) HBV FibroSURE®.
   d) HCV FibroSURE®.
2) For individuals with hepatitis C, hepatitis B, nonalcoholic fatty liver disease (NAFLD), **or** alcoholic hepatitis, the use of other multianalyte assays with algorithmic analysis (such as NASH FibroSURE®) **DOES NOT MEET COVERAGE CRITERIA.**
3) For individuals with liver disease not meeting the above criteria, the use of multianalyte assays with algorithmic analysis **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4) Except as previously described, the use of the following serum biomarkers in immunoassays and/or immunohistochemistry assays **DOES NOT MEET COVERAGE CRITERIA**:
   a) Signal-induced proliferation-associated 1 like 1 (SIPA1L1)
   b) microRNA (miRNA or miR) analysis, including but not limited to, the following:
      i) microRNA-21 (miRNA-21 or miR-21)
      ii) miRNA-29a (miR-29a)

iii) miRNA-122 (miR-122)

iv) miRNA-221 (miR-221)

v) miRNA-222 (miR-222)

c) Chitinase 3-like 1 (CHI3L1)

d) Hyaluronic acid

e) Type III procollagen (PCIII)

f) Type IV collagen

g) Laminin

h) Plasma caspase-generated cytokeratin-18

i) Micro-fibrillar associated glycoprotein 4 (MFAP4)

IV. Scientific Background

Fibrosis is a wound healing response in which damaged regions are encapsulated by an extracellular matrix. This is common in individuals with chronic liver injury but may be seen in other organs such as the kidneys or lungs. Chronic liver injury may be caused by numerous conditions, such as hepatitis, and progressive fibrosis may lead to cirrhosis (Friedman, 2020). Liver biopsy remains the gold standard for evaluation of chronic liver disease to monitor treatment and disease progression. However, this invasive procedure has several drawbacks including pain, bleeding, inaccurate staging due to sampling error, and variability of biopsy interpretation (Chin et al., 2016).

Serum biomarkers, such as the aspartate aminotransferase (AST) to platelet ratio (APRI), have been proposed as measures of hepatic fibrosis assessment, and numerous panels exist (Curry & Afdhal, 2019). These markers (and corresponding panels) may be categorized as "direct" or "indirect." Direct markers of fibrosis evaluate extracellular matrix turnover, and indirect markers signify changes in hepatic function. Direct biomarkers may be further subdivided by markers associated with matrix deposition, matrix degradation, or cytokines (and chemokines) associated with fibrogenesis. Procollagen I peptide, procollagen III peptide, type I collagen, type IV collagen, YKL-40 (chondrex), laminin, and hyaluronic acid, MMP-2, TIMP-1, -2, TGF-beta, TGF-alpha, and PDGF have all been proposed as direct measures of fibrosis. Indirect markers include serum aminotransferase levels, platelet count, coagulation parameters, gamma-glutamyl transferase (GGT), total bilirubin, alpha-2-macroglobulin, and alpha-2-globulin (haptoglobin) (Curry & Afdhal, 2019). Other markers have been investigated to be used independently or as part of these panels. The human microfibrillar-associated protein 4 (MFAP4) is located in extracellular matrix fibers and plays a role in disease-related tissue remodeling. Bracht et al. (2016) evaluated the "potential" of MFAP4 as a biomarker for hepatic fibrosis. A total of 542 patients were included, and the authors focused on differentiation of no to moderate (F0–F2) and severe fibrosis stages and cirrhosis (F3 and F4). In the "leave-one-out cross validation," a sensitivity of 85.8% and specificity of 54.9% was observed and the multivariate model yielded 81.3 % sensitivity and 61.5 % specificity. The authors suggested that "the combination of MFAP4 with existing tests might lead to a

more accurate non-invasive diagnosis of hepatic fibrosis and allow a cost-effective disease management in the era of new direct acting antivirals" (Bracht et al., 2016).

Plasma caspase-generated cytokeratin-18 fragments (CK-18) have been proposed as a biomarker in the diagnosis and staging of nonalcoholic steatohepatitis (NASH). Cusi et al. (2014) studied the clinical value of CK-18. The authors studied the adipose tissue, liver, and muscle insulin resistance of 424 patients as well as liver fat (n = 275) and histology (n = 318). The authors found that median CK-18 levels were elevated in patients with verses without nonalcoholic fatty liver disease (NAFLD) (209 U/L vs. 122 U/L) or with verses without NASH (232 U/L vs. 170 U/L). The CK-18 area under curve to predict NAFLD, NASH, or fibrosis were 0.77, 0.65, and 0.68, respectively. The overall sensitivity/specificity for NAFLD, NASH and fibrosis were 63%/83%, 58%/68% and 54%/85%, respectively. CK-18 correlated most strongly with ALT (r=0.57) and adipose tissue IR (insulin-suppreszsion of FFA: r=-0.43), but not with ballooning, body mass index, metabolic syndrome, or type 2 diabetes. The authors concluded, "Plasma CK-18 has a high specificity for NAFLD and fibrosis, but its limited sensitivity makes it inadequate as a screening test for staging NASH. Whether combined as a diagnostic panel with other biomarkers or clinical/laboratory tests may prove useful requires further study" (Cusi et al., 2014).

Likewise, Chitinase 3-like 1 (CHI3L1) has been proposed to be a better serum biomarker than hyaluronic acid, type III procollagen, type IV collagen, and laminin. CHI3L1 is preferentially expressed in hepatocytes over any other body tissue. Huang et al. (2015) investigated CHI3L1 in 98 patients with hepatitis B. The authors reported that CHI3L1 can be used to differentiate between early stages of liver fibrosis (S0-S2) from late stages (S3-S4) "with areas under the ROC curves (AUCs) of 0.94 for substantial (S2, S3, S4) fibrosis and 0.96 for advanced (S3, S4) fibrosis" (Huang et al., 2015). Wang et al. (2018) also report that CHI3L1 is a useful marker for the assessment of liver fibrosis before treatment, and can also be used to monitor change during therapy.

MicroRNA (miRNA) sequences have also been proposed as a marker of liver function. MiRNA sequences often have roles in gene regulation and other cellular processes, so changes in these sequences may indicate a liver condition (Tendler, 2020). For example, Abdel-Al et al. (2018) investigated miRNA's association with Hepatitis C virus (HCV) patients. Forty-two patients with HCV and early-stage fibrosis, 45 patients with HCV and late-stage fibrosis, and 40 healthy controls were examined and the expression patterns of 5 miRNA sequences (miR-16, miR-146a, miR-214-5p, miR-221, and miR-222) were measured. The authors found miRNA-222 to have the highest sensitivity and specificity for both fibrosis groups, and all mi-RNA sequences except miRNA-214-5p were significantly upregulated in fibrosis. MiRNA-221 was also found to have significant positive correlations with miRNA-16 and miRNA-146a. The authors concluded that "the high sensitivity and specificity of miRNA-222 and miRNA-221 in late-stage fibrosis indicate promising prognostic biomarkers for HCV-induced liver fibrosis (Abdel-Al et al., 2018).

Multiple biomarkers may be combined into a panel. Panels may include a combination of direct markers, indirect markers, or markers from both categories. The most studied panels are the aspartate aminotransferase (AST) to platelet ratio (APRI), FibroTest/FibroSure, and Hepascore, although many more exist. FibroTest/FibroSure incorporates alpha-2-macroglobulin, alpha-2-globulin (haptoglobin), gamma globulin, apolipoprotein A1, GGT, and total bilirubin, age, and sex. HepaScore measures bilirubin, GGT, hyaluronic acid, alpha-2-macroglobulin, age, and sex. These panels have demonstrated

some promising results, but Curry and Afdhal (2019) note that indeterminate outcomes are common. Furthermore, they state that no singular panel has emerged as the standard of care (Curry & Afdhal, 2018). Another test, known as the LIVERFASt™ by Fibronostics, utilizes a blood sample to measure 10 biomarkers; algorithm technology is used "to determine the fibrosis, activity and steatosis stages of the liver" (Fibronsotics, 2020).

Many combinations of biomarkers, and even combinations of panels, exist. For example, FibroMax combines FibroTest, SteatoTest, NashTest, ActiTest, and AshTest on the same result sheet and provides a more comprehensive estimation of the liver injury. This test measures 10 biomarkers which are as follows: GGT, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, alanine aminotransferase (ALT), AST Transaminase, triglycerides, cholesterol, and fasting glucose (BioPredictive, 2019). Fouad et al. (2013) analyzed samples from 44 patients and found that FibroMax results were positively correlated with viral load by quantitative polymerase chain reaction and histopathological findings. Further, body mass index was significantly higher in steatotic patients and was significantly associated with the results on FibroMax (Fouad et al., 2013).

Clinical Utility and Validity

Berends et al. (2007) performed a study assessing FibroTest's ability to detect methotrexate (MTX)-induced hepatic fibrosis. Twenty-four psoriasis patients that underwent a liver biopsy were included, and FibroTest identified 83 percent of the patients who had significant fibrosis. The authors suggested FibroTest may be used as part of monitoring MTX-induced fibrosis (Berends et al., 2007).

Kwok et al. (2014) performed a meta-analysis of non-invasive assessments of NASH. The authors identified 9 studies for transient elastography (TE) and 11 for cytokeratin-18 (CK-18). The pooled sensitivities and specificities for TE to diagnose F ≥ 2, F ≥ 3, and F4 disease were 79% and 75%, 85% and 85%, and 92% and 92%, respectively. CK-18 was found to have a pooled sensitivity of 66% and specificity of 82% in diagnosing NASH. The authors concluded that "At present, serum tests and physical measurements such as TE come close as highly accurate non-invasive tests to exclude advanced fibrosis and cirrhosis in NAFLD patients. CK18 has moderate accuracy in diagnosing NASH, while other biomarkers have not been extensively studied (Kwok et al., 2014)."

Gao et al. (2018) compared aspartate amino transferase–to-platelet ratio index (APRI), the Fibrosis-4 index (FIB-4), transient elastography (TE), and two-dimensional (2D) shear-wave elastography (SWE). A total of 402 patients with chronic hepatitis B were included. 2D-SWE was found to have the highest area under the curve (AUC), with 0.87 compared to APRI's 0.70, TE's 0.80, and FIB-4's 0.73 (Gao et al., 2018).

Dong et al. (2018) compared the performance of several biomarkers (serum hyaluronan (HA), procollagen type III N-terminal peptide (PIIINP), type IV collagen (IVC), laminin (LN), ALT, AST) to transient elastography (FibroScan). Seventy patients with hepatitis B underwent a liver biopsy. Fibrosis was found in 24 patients. The correlation of serum levels with fibrosis stage are as follows: 0.468 (HA), 0.392 (PIIINP), 0.538 (IVC), 0.213 (LN), 0.350 (ALT), 0.375 (AST). The authors found that the combination of all five biomarkers yielded a superior diagnostic performance (area under curve: 0.861) compared to all five alone (Dong et al., 2018).

A pilot study of the FM-fibro index was performed with 400 patients enrolled, and the FM-fibro index, CA-fibro index, and European Liver Fibrosis panel (ELF) were compared with respect to estimating prognosis of patients with NAFLD. Three separate biomarkers comprise the FM-fibro index: type IV collagen 7S, hyaluronic acid, and vascular cell adhesion molecule-1. The area under the curve was 0.7093 for the CA-fibro index, 0.7245 for ELF, and 0.7178 (type IV collagen 7S)/0.7095 (hyaluronic acid)/0.7065 (vascular cell adhesion molecule-1) (Itoh et al., 2018). The sensitivity and specificity of the FM-fibro index for predicting NASH-related fibrosis was 0.5359/0.5210/0.4641 and 0.8333/0.8182/0.8788, respectively (Itoh et al., 2018). The accuracy of the FM-fibro index was not significantly different from that of the CA-fibro index and the ELF panel.

Patel et al. (2018) performed a retrospective study focusing on fibrosis scoring systems to identify NAFLD. A total of 329 patients (296 NAFLD, 33 controls) were included. The following indices were studied: "NAFLD fibrosis score (NFS), fibrosis-4 calculator (FIB-4), aspartate aminotransferase-to-alanine aminotransferase ratio (AST/ALT ratio), AST-to-platelet ratio index (APRI), and body mass index, AST/ALT ratio, and diabetes (BARD) score by age groups" (Patel et al., 2018). NFS and FIB-4 were found to best predict advanced fibrosis with areas under curve of 0.71-0.76 and 0.62-0.80 respectively. However, the authors concluded that "While NFS and FIB-4 scores exhibit good diagnostic accuracy, FIB-4 is optimal in identifying NAFLD advanced fibrosis in the VHA. Easily implemented as a point-of-care clinical test, FIB-4 can be useful in directing patients that are most likely to have advanced fibrosis to GI/hepatology consultation and follow-up" (Patel et al., 2018).

Kim et al. (2017) evaluated the "association between plasma miR-122 [microRNA-122] and treatment outcomes following transarterial chemoembolization (TACE) in hepatocellular carcinoma patients." A total of 177 patients were included, and miR-122 levels were measured; the researchers found that 112 patients exhibited TACE refractoriness. Multivariate analyses showed that tumor number (hazard ratio [HR], 2.51) and tumor size (HR, 2.65) can independently predict overall TACE refractoriness. High miR-122 expression (> 100) was associated with early TACE refractoriness (within 1 year; HR, 2.77; 95% CI,) together with tumor number (HR, 22.73) and tumor size (HR, 4.90). Univariate analyses showed that high miR-122 expression tends to be associated with poor liver transplantation-free survival (HR, 1.42). However, this was statistically insignificant in multivariate analysis. The authors concluded that "High expression levels of plasma miR-122 are associated with early TACE refractoriness in HCC patients treated with TACE" (Kim et al., 2017).

Suehiro et al. (2018) performed a study analyzing "the importance of serum exosomal miRNA expression levels in hepatocellular carcinoma (HCC) patients that underwent transarterial chemoembolization (TACE)." Seventy-five patients underwent TACE. Exosomal miR-122 expression levels significantly decreased after TACE. The expression levels of exosomal miR-122 before TACE were shown to correlate significantly with AST (r=0.31) and ALT (r=0.33) levels. According to the median relative expression of miR-122 after TACE/before TACE (miR-122 ratio) in liver cirrhosis patients (n=57), the patients with a higher miR-122 ratio had significantly longer disease-specific survival compared with that of the patients with the lower miR-122 ratio. A lower exosomal miR-122 ratio (HR 2.720) was associated with the disease-specific survival. The authors concluded that "the exosomal miR-122 level

alterations may represent a predictive biomarker in HCC patients with liver cirrhosis treated with TACE" (Suehiro et al., 2018).

Kar et al. (2019) analyzed the performance of biomarkers implicated in hepatic inflammation. The authors enrolled 52 patients with NAFLD/NASH and evaluated the following biomarkers: IL-6, CRP, TNFα, MCP-1, MIP-1β, eotaxin, and VCAM-1. Serum IL-6 was found to be increased in patients with advanced fibrosis (2.71 pg/mL in fibrosis stages 3 and 4 compared to 1.26 pg/mL in stages 1-2 and 1.39 pg/mL in stage 0), but there were no other significant differences in CRP, TNFα, MCP-1, MIP-1β. VCAM-1 was noted to have increased by 55% over the mild fibrosis group and 40% over the no fibrosis group. VCAM-1 was also observed to have an area under curve of 0.87. The authors suggested that the "addition of biomarkers such as IL-6 and VCAM-1 to panels may yield increased sensitivity and specificity for staging of NASH" (Kar et al., 2019).

Srivastava et al. (2019) performed a cost-benefit analysis of non-invasive fibrosis tests (NILTS) for nonalcoholic fatty liver disease (NAFLD). The authors compared the current standard of care, FIB-4, and the Enhanced Liver Fibrosis (ELF) panel. The simulations consisted of 10000 NAFLD patients. Standard care (SC) was compared to the following four scenarios: "FIB-4 for all patients followed by ELF test for patients with indeterminate FIB-4 results; FIB-4 followed by fibroscan for indeterminate FIB-4; ELF alone; and fibroscan alone." The authors identified the following observations: "Introduction of NILT increased detection of advanced fibrosis over 1 year by 114, 118, 129 and 137% compared to SC in scenarios 2, 3, 4 and 5 respectively with reduction in unnecessary referrals by 85, 78, 71 and 42% respectively. Total budget spend [sic] was reduced by 25.2, 22.7, 15.1 and 4.0% in Scenarios 2, 3, 4 and 5 compared to £670 K at baseline." The authors suggested that the "use of NILT in primary care can increase early detection of advanced liver fibrosis and reduce unnecessary referral of patients with mild disease and is cost efficient" (Srivastava et al., 2019).

Weis et al. (2019) evaluated miRNA expression's ability to distinguish between HCC and cirrhosis. Sixty patients with chronic hepatitis C (CHC) were divided into three groups; 20 with fibrosis stages 0-2, 20 with cirrhosis, and 20 with cirrhosis and HCC. A total of 372 miRNA sequences were measured. The authors found that a theoretical panel consisting of miRNA-122-5p, miRNA-486-5p, and miRNA-142-3p distinguished HCC from cirrhosis (area under the curve [AUC]= 0.94; sensitivity = 80%, specificity = 95%) outperforming alpha-fetoprotein (AFP) (AUC = 0.64). Another theoretical panel of miRNA-122-5p and miRNA-409-3p distinguished cirrhosis from mild disease (AUC = 0.80; sensitivity = 85%, specificity = 70%). The authors concluded that "MicroRNAs have great potential as diagnostic biomarkers in CHC, particularly in HCC where they outperform the only currently-used biomarker, AFP" (Weis et al., 2019).

Both Parikh et al. (2017) and Kaswala et al. (2016) performed studies evaluating the diagnostic accuracy of non-invasive markers for liver conditions. Parikh et al. (2017) focused on chronic hepatitis B virus (HBV) infections while Kaswala et al. (2016) studied nonalcoholic fatty liver. Tables detailing their summarized findings are listed below:

Diagnostic accuracy of most commonly used non-invasive fibrosis (≥F2) tests in chronic HBV infection from (Parikh et al., 2017)				
Test	**Cut-off**	**AUROC**	**Sensitivity (%)**	**Specificity (%)**
Indirect markers				
FIB-4 index (high cut-off)	3.25	N/A	16.2	73.6
FIB-4 index (low cut-off)	1.45–1.62	0.78	65	77
APRI (low cut-off)	0.5	0.79	84	41
APRI (high cut-off)	1.5		49	84
Forns index (low cut-off)	3.11	0.68	91.4	31.5
Forns index (high cut-off)	5.11	N/A	42.5	75
Direct markers				
Hyaluronic acid	113–203	0.73	63–80	78–94
Hepascore	0.32	0.75	74	69
Fibrotest	0.38	0.77	65	78
Fibrometer	0.47	0.84	73	80
ELF	8.75	0.8	NA	NA
Diagnostic accuracy of most commonly used non-invasive fibrosis tests in nonalcoholic fatty liver (NAFL) from (Kaswala et al., 2016)				
Test	**Cut-off**	**AUROC**	**Sensitivity (%)**	**Specificity (%)**
AST/ALT ratio	1	0.83	21	90
AST to platelet ratio index (low cutoff)	0.45	0.67–0.94	30	93
AST to platelet ratio index (high cutoff)	1.5			
BAAT score	2	0.84	71	80
BARD	2	0.8	86.8	32.5
ELF test	8.5–11.35	0.82–0.90	80	90
FibroMeter (low cutoff)	F3: 0.61	0.90–0.94	81	84
FibroMeter (high cutoff)	0.71			
FibroTest (low cutoff)	0.3	0.81–0.92	15–77	77–90
FibroTest (high cutoff)	0.7			
FIB-4 (low cutoff)	1.3–1.92	0.88	26–74	71–98
FIB-4 (high cutoff)	3.25			
Hepascore	0.37	0.81	75.5	84.1
	0.7	0.9	87	89
NAFLD (low cutoff)	−1.45	0.81	51	96
NAFLD (high cutoff)	0.67			

AST- aspartate aminotransferase; APRI- AST to platelet ratio; BAAT- body mass index (BMI), age, alanine aminotransferase (ALT), triglycerides; BARD- BMI, AST/ALT ratio, diabetes; ELF- Enhanced Liver Fibrosis panel; FIB-4- Fibrosis-4 index; NAFLD – Nonalcoholic fatty liver disease

Bril et al. (2019) assessed the performance of the FibroTest, along with other tests which measure steatosis, necrosis, and inflammation (the SteatoTest, ActiTest, NashTest), in a cohort of patients with type 2 diabetes. A total of 220 diabetic patients participated in this study. Plasma samples from each participant were used for the FibroTest. The researchers note that "Regarding the FibroTest score, its performance to identify patients with moderate or advanced fibrosis was 0.67" (Bril et al., 2019). The authors concluded that "Non-invasive panels for the diagnosis of steatosis, NASH and/or fibrosis, which were developed and validated in non-diabetic cohorts, underperformed when applied to a large cohort of patients with T2DM [type 2 diabetes mellitus]" (Bril et al., 2019)

In a metanalysis, 7 studies reported the accuracy of FibroTest™ in nonalcoholic fatty liver disease (NAFLD) patients. The mean AUC was 0.77, mean sensitivity was 0.72, and mean specificity was 0.69. Due to poor AUC, sensitivity, and specificity values, FibroTest™ did not meet the minimally acceptable performance level in detecting significant, advanced, or any fibrosis. However, diagnostic accuracy of FibroTest™ was more promising in detecting cirrhosis, with an AUC of 0.92. The author states that in primary care settings which have a low disease prevalence, FibroTest™ can have a high negative predictive value, based on sensitivities between 0.90 and 0.98, demonstrating its ability to rule out advanced fibrosis in NAFLD patients. However, the test does have low specificity, leading to a considerable number of false positive results, which can lead to invasive and expensive follow-up tests. Overall, "this analysis showed that by optimizing sensitivity to values above 0.90, the test could result in high NPVs (>90%) in settings with low prevalence of disease, such as primary and secondary care settings, but with relatively low PPVs (11–61%)" (Vali et al., 2021).

V. Guidelines and Recommendations

American Association for the Study of Liver Diseases (AASLD)

The 2015 AASLD and Infectious Diseases Society of America (IDSA) recommendations for testing, managing, and treating adults infected with hepatitis C virus stated that "Recently, noninvasive tests to stage the degree of fibrosis in patients with chronic HCV infection include models incorporating indirect serum biomarkers (routine tests such as aspartate transaminase, alanine transaminase [ALT], and platelet count), direct serum biomarkers (components of the extracellular matrix produced by activated hepatic stellate cells), and vibration-controlled transient liver elastography. No single method is recognized to have high accuracy alone, and the results of each test must be interpreted carefully." The guidelines further stated that "although liver biopsy is the diagnostic standard, sampling error and observer variability limit test performance, particularly when inadequate sampling occurs. In addition, the test is invasive and minor complications are common, limiting patient and practitioner acceptance. Serious complications such as bleeding, although rare, are well recognized" (AASLD-IDSA, 2015).

The 2018 AASLD and Infectious Diseases Society of America (IDSA) recommendations for HCV testing stated that "evaluation for advanced fibrosis using liver biopsy, imaging, and/or noninvasive markers is recommended for all persons with HCV infection, to facilitate an appropriate decision regarding HCV treatment strategy and to determine the need for initiating additional measures for the management of cirrhosis (e.g., hepatocellular carcinoma screening). Rating: Class I, Level A" (AASLD-IDSA, 2018).

The 2018 AASLD update (Terrault et al., 2018) on prevention, diagnosis and treatment of chronic hepatitis B state that:

For monitoring patients with a chronic HBV infection, who are not currently on treatment, "Alternative methods to assess fibrosis are elastography (preferred) and liver fibrosis biomarkers (e.g., FIB-4 or FibroTest). If these noninvasive tests indicate significant fibrosis (≥F2), treatment is recommended."

The 2018 AASLD practice guidelines (Chalasani et al., 2017) on the diagnosis and management of nonalcoholic fatty liver disease recommend:

- "In patients with NAFLD, metabolic syndrome predicts the presence of steatohepatitis, and its presence can be used to target patients for a liver biopsy."
- "NFS or FIB-4 index are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis (stage 3) or cirrhosis (stage 4)."
- "Vibration controlled transient elastography or magnetic resonance elastography are clinically useful tools for identifying advanced fibrosis in patients with NAFLD."

The AASLD does not mention miRNA for assessment in liver disease.

A 2019 update from the AASLD and IDSA states that "Noninvasive tests using serum biomarkers or imaging allow for accurate diagnosis of cirrhosis in most individuals" and frequently used noninvasive methods to estimate liver disease severity include "serum fibrosis marker panels" (AASLD-IDSA, 2019). Further, regarding recommendations for counseling persons with an active HCV infection, the guideline recommend that "Evaluation for advanced fibrosis using noninvasive markers or liver biopsy, if required, is recommended for all persons with HCV infection to facilitate an appropriate decision regarding HCV treatment strategy, and to determine the need for initiating additional measures for cirrhosis management (eg, hepatocellular carcinoma screening)" (AASLD-IDSA, 2019).

In a 2021 update, AASLD discussed changes in liver biochemistry during normal pregnancy. AASLD states that an "elevation in aminotransferases, bilirubin, or bile acids in pregnancy is abnormal and requires investigation. Evaluation in pregnant patients must include a thorough history (including travel, environmental, and drug exposures), physical examination, and focused serologic testing. Hepatic ultrasonography (US) is the favored initial imaging modality. Diagnosis can usually be determined without liver biopsy" (Sarkar et al., 2021).

American Gastroenterological Association (AGA)

The 2017 guidelines (Lim et al., 2017) on the Role of Elastography in the Evaluation of Liver Fibrosis state that:

- "In patients with chronic hepatitis C, the AGA recommends vibration controlled transient elastography, if available, rather than other nonproprietary, noninvasive serum tests (APRI, FIB-4) to detect cirrhosis."

- "In patients with chronic hepatitis B, the AGA suggests vibration controlled transient elastography (VCTE) rather than other nonproprietary noninvasive serum tests (ie, APRI and FIB-4) to detect cirrhosis."
- "The AGA makes no recommendation regarding the role of VCTE in the diagnosis of cirrhosis in adults with NAFLD."

World Health Organization (WHO)

In March 2015, the WHO released Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. In the section titled "Non-invasive Assessment of Liver Disease Stage at Baseline and during Follow up," the following is noted: aspartate aminotransferase (AST)-to-platelet ratio index (APRI) is recommended as the preferred non-invasive test (NIT) to assess for the presence of cirrhosis (APRI score >2 in adults) in resource-limited settings. Transient elastography (e.g., FibroScan) or FibroTest may be the preferred NITs in settings where they are available and cost is not a major constraint (WHO, 2015).

The WHO also published guidelines for management of patients with Hepatitis C. In it, they suggest "that aminotransferase/platelet ratio index (APRI) or FIB-4 be used for the assessment of hepatic fibrosis rather than other non-invasive tests that require more resources such as elastography or FibroTest." However, they do note that "FibroScan, which is more accurate than APRI and FIB-4, may be preferable in settings where the equipment is available and the cost of the test is not a barrier to testing."

The WHO does not mention miRNA as a tool for assessment of hepatitis (WHO, 2018).

US Preventive Services Task Force (USPSTF)

The USPSTF published their final recommendation statement on Hepatitis C screening in adolescents and adults in 2020. THE USPSTF recommends "screening for hepatitis C virus (HCV) in adults aged 18 to 79" (grade B recommendation) (USPSTF, 2020).

National Institute for Health and Care Excellence (NICE)

NICE has released guidelines regarding chronic liver conditions. They note that the enhanced liver fibrosis test (ELF) may be considered in patients with NAFLD to test for advanced liver fibrosis (NICE, 2016).

European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity

These joint guidelines include recommendations for fibrosis, mentioning ELF, FibroTest, NFS, and FIB-4. Their recommendations include the following:

- "Biomarkers and scores of fibrosis, as well as transient elastography, are acceptable non-invasive procedures for the identification of cases at low risk of advanced fibrosis/cirrhosis (A2). The combination of biomarkers/ scores and transient elastography might confer additional diagnostic accuracy and might save a number of diagnostic liver biopsies (B2)."
- "Monitoring of fibrosis progression in clinical practice may rely on a combination of biomarkers/scores and transient elastography, although this strategy requires validation (C2)."

- "The identification of advanced fibrosis or cirrhosis by serum biomarkers/scores and/or elastography is less accurate and needs to be confirmed by liver biopsy, according to the clinical context (B2)."
- The guidelines observe that due to non-invasive tests' high negative predictive values, they "may be confidently used for first-line risk stratification to exclude severe disease." Still, they state that "There is no consensus on thresholds or strategies for use in clinical practice when trying to avoid liver biopsy. Some data suggest that the combination of elastography and serum markers performs better than either method alone. Importantly, longitudinal data correlating changes in histological severity and in non-invasive measurements are urgently needed."
- For nonalcoholic steatohepatitis (NASH), the guidelines state that "to date, non-invasive tests are not validated for the diagnosis of NASH" and addresses CK-18 as a proposed biomarker.
- For monitoring of NAFLD, the guidelines state that "Monitoring should include routine biochemistry, assessment of comorbidities and non-invasive monitoring of fibrosis" (EASL, 2016).

[1]Grade A Evidence Quality- High: Further research is very unlikely to change our confidence in the estimate of effect

[2]Grade B Evidence Quality- Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate

[3]Grade C Evidence Quality- Low or very low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and may change the estimate effect. Any estimate of effect is uncertain.

[4] Grade 1 Recommendation- Strong: Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes, and cost

[5]Grade 2 Recommendation- Weak: Variability in preferences and values, or more uncertainty. Recommendation is made with less certainty, higher cost or resource consumption

The EASL also released guidelines on management of Hepatitis C. In it, they recommend that "Fibrosis stage must be assessed by non-invasive methods initially, with liver biopsy reserved for cases where there is uncertainty or potential additional aetiologies." A1[1,4])" (grading scale same as the 2016 guideline above). Non-invasive methods include FibroScan, ARFI, Aixplorer, FibroTest, APRI, and FIB-4 (EASL, 2018).

Guidelines for Hepatitis B were also published. In it, EASL remarks that "the diagnostic accuracy of all non-invasive methods is better at excluding than confirming advanced fibrosis or cirrhosis." Non-invasive methods include assessment of serum biomarkers of liver fibrosis (EASL, 2017).

The EASL also published guidelines titled "Non-invasive tests for evaluation of liver disease severity and prognosis." In it, they state the following (grading scale same as the 2016 guideline above):

- "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests. (A1[1,4])"
- "Serum biomarkers of fibrosis are well validated in patients with chronic viral hepatitis (with more evidence for HCV than for HBV and HIV/HCV coinfection). They are less well validated in NAFLD and not validated in other chronic liver diseases. (A1[1,4])"
- "Their performances are better for detecting cirrhosis than significant fibrosis. (A1[1,4])"
- "FibroTest®, APRI and NAFLD fibrosis score are the most widely used and validated patented and nonpatented tests. (A1[1,4])"
- "Among the different available strategies, algorithms combining TE and serum biomarkers appear to be the most attractive and validated one. (A1[1,4])"
- "HCV patients who were diagnosed with cirrhosis based on non-invasive diagnosis should undergo screening for HCC and PH and do not need confirmatory liver biopsy. (A1[1,4])"
- "Non-invasive assessment including serum biomarkers or TE can be used as first line procedure for the identification of patients at low risk of severe fibrosis/ cirrhosis. (A1[1,4])"
- "The identification of significant fibrosis is less accurate with non-invasive tests as compared to liver biopsy and may necessitate, according to the clinical context, histological confirmation. (A1[1,4])"
- "Follow-up assessment by either serum biomarkers or TE for progression of liver fibrosis should be performed among NAFLD patients at a 3 year interval (EASL & ALEH, 2015) (A1[1,4])"

[1]Grade A Evidence Quality- High: Further research is very unlikely to change our confidence in the estimate of effect

[2]Grade B Evidence Quality- Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate

[3]Grade 1 Recommendation- Strong: Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes, and cost

[4]Grade 2 Recommendation- Weak: Variability in preferences and values, or more uncertainty. Recommendation is made with less certainty, higher cost or resource consumption

EASL released guidelines on non-invasive tests for evaluation of liver disease severity and prognosis (EASL, 2020). The following recommendations were made (grading scale same as the 2016 guideline above):

- "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests (A1[1,4])"
- "TE and serum biomarkers have equivalent performance for detecting significant fibrosis in patients with untreated viral hepatitis (A1[1,4])"

- "In patients with viral hepatitis C, when TE and serum biomarkers results are in accordance, the diagnostic accuracy is increased for detecting significant fibrosis but not for cirrhosis. In cases of unexplained discordance, a liver biopsy should be performed if the results would change the patient management (A1[1,4])"
  "All HCV patients should be screened to exclude cirrhosis by TE if available. Serum biomarkers can be used in the absence of TE (A1[1,4])"(EASL, 2020).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81596	Infectious disease, chronic hepatitis c virus (HCV) infection, six biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, and haptoglobin) utilizing serum, prognostic algorithm reported as scores for fibrosis and necroinflammatory activity in liver Proprietary test: HCV FibroSURE™, FibroTest™ Laboratory/Manufacturer: BioPredictive S.A.S
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
0002M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, haptoglobin, AST, glucose, total cholesterol and triglycerides) utilizing serum, prognostic algorithm reported as quantitative scores for fibrosis, steatosis and alcoholic steatohepatitis (ASH) Proprietary test: ASH FibroSURE™ Laboratory/Manufacturer: BioPredictive S.A.S
0003M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, haptoglobin, AST, glucose, total cholesterol and triglycerides) utilizing serum, prognostic algorithm reported as quantitative scores for fibrosis, steatosis and nonalcoholic steatohepatitis (NASH) Proprietary test: NASH FibroSURE™ Laboratory/Manufacturer: BioPredictive S.A.S

0014M	Liver disease, analysis of 3 biomarkers (hyaluronic acid [HA], procollagen III amino terminal peptide [PIIINP], tissue inhibitor of metalloproteinase 1 [TIMP-1]), using immunoassays, utilizing serum, prognostic algorithm reported as a risk score and risk of liver fibrosis and liver-related clinical events within 5 years Proprietary test: Enhanced Liver Fibrosis™ (ELFTM) Test Lab/Manufacturer: Siemens Healthcare Diagnostics Inc/Siemens Healthcare Laboratory LLC
0166U	Liver disease, 10 biochemical assays (α2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT, AST, triglycerides, cholesterol, fasting glucose) and biometric and demographic data, utilizing serum, algorithm reported as scores for fibrosis, necroinflammatory activity, and steatosis with a summary interpretation Proprietary test: LiverFASt™ Lab/Manufacturer: Fibronostics
0344U	Hepatology (nonalcoholic fatty liver disease [NAFLD]), semiquantitative evaluation of 28 lipid markers by liquid chromatography with tandem mass spectrometry (LC-MS/MS), serum, reported as at-risk for nonalcoholic steatohepatitis (NASH) or not NASH

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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Serum Tumor Markers for Malignancies

Policy #: AHS – G2124	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/22/22, 10/27/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Tumor biomarkers are proteins detected in the blood, urine, or other body fluids that are either produced by the tumor itself or in response to its presence used to help detect, diagnose, and manage some types of cancer (Hottinger & Hormigo, 2011).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

Note: Except for where otherwise specified in the coverage criteria below, quarterly measurement of designated serum tumor markers is permitted for follow-up, monitoring, and/or surveillance

1) Measurement of the following serum tumor markers **MEETS COVERAGE CRITERIA** for the following indications:
   a) Acute lymphoblastic leukemia (ALL) and pediatric acute lymphoblastic leukemia (PED-ALL)

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

b) Acute myeloid leukemia (AML)
   i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
c) B-cell lymphoma
   i) **Beta-2 microglobulin (B2M)**: initial diagnostic evaluation
   ii) **Serum light chains** (Castleman disease only): initial diagnostic evaluation
   iii) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
d) Bone neoplasms (metastatic and primary)
   i) **Alkaline Phosphatase (ALP)**: initial diagnostic evaluation
   ii) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
e) Breast cancer (metastatic)
   i) **Cancer Antigen 15-3 and 27.29 (CA 15-3 and 27.29)**: monitoring
   ii) **Carcinoembryonic Antigen (CEA)**: monitoring
f) Breast implant-associated anaplastic large cell lymphoma (ALCL)
   i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation and staging
g) Chronic lymphocytic leukemia/small lymphocytic lymphoma
   i) **Beta-2 microglobulin (B2M)**: initial diagnostic evaluation
   ii) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
h) Colon cancer
   i) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation and post-treatment surveillance every 3-6 months for 2 years, then every 6 months for a total of 5 years
i) Endometrial cancer
   i) **Cancer Antigen 125 (CA-125)**: additional diagnostic evaluation and/or surveillance
j) Epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer:
   i) initial diagnostic evaluation, during primary chemotherapy, and/or monitoring for complete response:
      (a) **Alpha fetoprotein (AFP)**
      (b) **Beta human chorionic gonadotropin (beta-hCG)**
      (c) **Cancer Antigen 19-9 (CA 19-9)**
      (d) **Cancer Antigen 125 (CA-125)**
      (e) **Carcinoembryonic Antigen (CEA)**
      (f) **Inhibin (INHA) expression**
      (g) **Lactate dehydrogenase (LDH)**
k) Extrahepatic cholangiocarcinoma
   i) **Cancer Antigen 19-9 (CA 19-9)**: initial diagnostic evaluation
   ii) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation
l) Gallbladder cancer

i) **Cancer Antigen 19-9 (CA 19-9)**: initial or postoperative diagnostic evaluation and/or surveillance
ii) **Carcinoembryonic Antigen (CEA)**: initial or postoperative diagnostic evaluation and/or surveillance

m) Hairy cell leukemia
   i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
n) Hepatocellular carcinoma
   i) **Alpha fetoprotein (AFP)**: initial diagnostic evaluation and screening and/or surveillance (every 3-6 months for 2 years, then every 6 months up to 5 years)
   ii) **Cancer Antigen 19-9 (CA 19-9)**: initial diagnostic evaluation
o) Hodgkin lymphoma
   i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
p) Intrahepatic cholangiocarcinoma
   i) **Alpha fetoprotein (AFP)**: initial diagnostic evaluation
   ii) **Cancer Antigen 19-9 (CA 19-9)**: initial diagnostic evaluation
   iii) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation
q) Kidney cancer
   i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
r) Less common ovarian cancers
   i) Mucinous Carcinoma of the Ovary: initial diagnostic and (if necessary) additional evaluations
      (a) **Cancer Antigen 19-9 (CA 19-9)**
      (b) **Carcinoembryonic Antigen (CEA)**
   ii) Ovarian low malignant potential tumors (borderline ovarian epithelial tumors): monitoring/follow-up every 3–6 months for up to 5 years, then annually
      (a) **Alpha fetoprotein (AFP)**
      (b) **Beta human chorionic gonadotropin (beta-hCG)**
      (c) **Cancer Antigen 19-9 (CA 19-9)**
      (d) **Cancer Antigen 125 (CA-125)**
      (e) **Carcinoembryonic Antigen (CEA)**
      (f) **Inhibin (INHA) expression**
      (g) **Lactate dehydrogenase (LDH)**
   iii) Malignant germ cell tumors: surveillance no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5
      (a) **Alpha fetoprotein (AFP)**
      (b) **Beta human chorionic gonadotropin (beta-hCG)**
      (c) **Cancer Antigen 19-9 (CA 19-9)**
      (d) **Cancer Antigen 125 (CA-125)**
      (e) **Carcinoembryonic Antigen (CEA)**

(f) **Inhibin (INHA) expression**

(g) **Lactate dehydrogenase (LDH)**

iv) Malignant sex cord stromal tumors: surveillance based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)

(a) **Alpha fetoprotein (AFP)**

(b) **Beta human chorionic gonadotropin (beta-hCG)**

(c) **Cancer Antigen 19-9 (CA 19-9)**

(d) **Cancer Antigen 125 (CA-125)**

(e) **Carcinoembryonic Antigen (CEA)**

(f) **Inhibin (INHA) expression**

(g) **Lactate dehydrogenase (LDH)**

s) Medullary carcinoma

i) **Calcitonin (CALCA) expression**: initial diagnostic evaluation, monitoring, and/or surveillance 2-3 months postoperative, then every 6-12 months

ii) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation and surveillance 2-3 months postoperative, then every 6-12 months

t) Melanoma (cutaneous)

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation for metastatic or recurrent disease

u) Melanoma (uveal)

i) **Alkaline phosphatase (ALP)**: initial diagnostic evaluation for metastatic or recurrent disease

ii) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation for metastatic or recurrent disease

v) Multiple myeloma

i) **Beta-2 microglobulin (B2M)**: initial diagnostic evaluation, staging, and/or follow-up/surveillance as needed

ii) **Serum free light chain**: initial diagnostic evaluation and/or surveillance as needed

iii) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation, staging, and/or follow-up/surveillance as needed

w) Myelodysplastic syndromes

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

x) Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase fusion genes

i) **Tryptase**: initial diagnostic evaluation

y) Myeloproliferative neoplasms

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation and/or monitoring while on and after therapy

z) Neuroendocrine and adrenal tumors - multiple endocrine neoplasia, type 2

i) **Calcitonin (CALCA) expression**: initial diagnostic evaluation

ii) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation

aa) Occult primary mass of the liver, mediastinum, or retroperitoneum

i) **Alpha fetoprotein (AFP)**: initial diagnostic evaluation

bb) Occult primary mass of the mediastinum or retroperitoneum

i) **Alpha fetoprotein (AFP)**: additional diagnostic evaluation

ii) **Beta human chorionic gonadotropin (beta-hCG)**: initial diagnostic evaluation

cc) Occult primary adenocarcinoma or carcinoma not otherwise specified

i) **Cancer Antigen 125 (CA-125)**: additional diagnostic evaluation (in those with a uterus and/or ovaries present)

dd) Pancreatic adenocarcinoma

i) **Cancer Antigen 19-9 (CA 19-9)**: initial diagnostic evaluation, risk classification, monitoring, and/or surveillance (every 3-6 months for 2 years, then every 6-12 months as clinically indicated)

ee) Pediatric aggressive mature B-cell lymphomas

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

ff) Peritoneal mesothelioma (malignant)

i) **Cancer Antigen 125 (CA-125)**: initial diagnostic evaluation

gg) Primary cutaneous lymphomas

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

hh) Rectal cancer

i) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation, monitoring, and/or surveillance every 3-6 months for 2 years, then every 6 months for a total of 5 years

ii) Richter's syndrome

i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

jj) Saccrococcygeal teratoma

i) **Alpha fetoprotein (AFP)**: initial diagnostic evaluation and surveillance for up to 3 years

ii) **Beta human chorionic gonadotropin (beta-hCG)**: initial diagnostic evaluation

kk) Small bowel adenocarcinoma

i) **Cancer Antigen 19-9 (CA 19-9)**: initial diagnostic evaluation and/or surveillance (every 3-6 months for 2 years, then every 6 months for a total of 5 years)

ii) **Carcinoembryonic Antigen (CEA)**: initial diagnostic evaluation and/or surveillance (every 3-6 months for 2 years, then every 6 months for a total of 5 years)

ll) Small cell lung cancer

i) **Lactate dehydrogenase (LDH)**: prognosis

mm) Systemic light chain amyloidosis

i) **Alkaline Phosphatase (ALP)**: initial diagnostic evaluation

ii) **B-type natriuretic peptide (BNP) or N-terminal fragment of B-type natriuretic peptide (NT-proBNP)**: initial diagnostic evaluation and staging

iii) **Beta-2 microglobulin (B2M)**: initial diagnostic evaluation

iv) **Serum free light chain**: initial diagnostic evaluation

v) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation
vi) **Troponin T**: initial diagnostic evaluation and staging

nn) Systemic mastocytosis
i) **Tryptase**: initial diagnostic evaluation, monitoring response to therapy, and/or risk classification

oo) T-cell lymphomas
i) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

pp) Testicular cancer (nonseminoma and pure seminoma):
i) Initial and post diagnostic evaluation, staging, risk classification, post-treatment follow-up, and surveillance:
(a) **Alpha fetoprotein (AFP)**
(b) **Beta human chorionic gonadotropin (beta-hCG)**
(c) **Lactate dehydrogenase (LDH)**

qq) Thymomas and thymic carcinomas
i) **Alpha fetoprotein (AFP)**: initial diagnostic evaluation
ii) **Beta human chorionic gonadotropin (beta-hCG)**: initial diagnostic evaluation

rr) Undiagnosed pelvic mass
i) **Inhibin (INHA) expression**: initial diagnostic evaluation for clinical indication to assess for LCOC (Less Common Ovarian Cancers) and pregnancy

ss) Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma
i) **Beta-2 microglobulin (B2M)**: initial diagnostic evaluation and prognostication at the time of first-line treatment initiation
ii) **Serum free light chain**: initial diagnostic evaluation
iii) **Lactate dehydrogenase (LDH)**: initial diagnostic evaluation

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other cancer indications not discussed above, use of the above biomarkers (alone or in a panel of serum tumor markers) **DOES NOT MEET COVERAGE CRITERIA.**

3) All other serum tumor markers not addressed above (alone or in a panel of serum tumor markers) **DO NOT MEET COVERAGE CRITERIA.**

4) For the screening and detection of cancer, analysis of proteomic patterns in serum **DOES NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Actionable molecular assays for tumor biomarkers may guide treatment decisions for common malignancies (Febbo et al., 2011). Tumor biomarkers are proteins detected in blood, urine or body fluids that serve as surrogate indicators to increase or decrease the clinician's suspicion of future clinically important events. These can be used to determine risk, screen for early cancers, establish diagnosis, estimate prognosis, predict that a specific therapy will work, and/or monitor for disease recurrence or progression (Catharine M. Sturgeon et al., 2008). The National Comprehensive Cancer Network (NCCN) task force guidelines recommend that tumor markers be classified by indication as diagnostic, prognostic, predictive and companion tests. An individual marker may serve more than one purpose and thus can fall into more than one category of biomarker. Biomarkers may also have different categorization across different stages of disease or different types of tumors (Febbo et al., 2011). Some of these categories are listed below:

- Diagnostic biomarkers – Tumor biomarkers that aid in the diagnosis or subclassification of a particular disease state. Detection of diagnostic biomarkers may result in different management of the disease, but the marker is used primarily to establish that a particular disease is present in the patient sample. An example of a diagnostic biomarker is the Philadelphia chromosome in chronic myelogenous leukemia.
- Prognostic – Tumor biomarkers that have an association with some clinical outcomes, such as overall survival or recurrence-free survival, independent of the treatment rendered. An example is the p53 gene, whose presence may indicate a more aggressive type of cancer.
- Predictive - Tumor biomarkers predict the activity of a specific class or type of therapy and are used to help make more specific treatment decisions. An example is human epidermal growth factor 2 (HER2), which is assessed in breast cancer patients. Patients who are negative for this biomarker do not respond as well to trastuzumab.
- Companion - Biomarkers may be diagnostic, prognostic, or predictive, but are used to identify a subgroup of patients for whom a therapy has shown benefit. This category of biomarker is similar to the predictive category, but these biomarkers do not usually have independent prognostic or predictive strength (Febbo et al., 2011).

Proprietary Testing

There are laboratory developed tests for serum tumor markers, but the clinical validity of these test have not been clearly proven yet.

The IMMray® PanCan-d test measures nine serum biomarkers, including immunoregulatory and tumor biomarkers. The test uses IMMray microarray technology that prints single chain fragment antibodies onto a slide. The slide is preloaded with a microarray of antibodies. The slide can then be screeded to measure the serum response to each biomarker. In a blind validation study, the test was reported to have a 99% specificity and 89% sensitivity rate when identifying stages I and II PDAC (n=56) versus high-risk

individual controls. The accuracy increased to 92% sensitivity and 99% specificity across stage I-IV PDAC (n=157) (Immunovia, 2023).

BeScreened™–CRC is a colorectal cancer screening test. BeScreened™–CRC tests three blood-based proteins that are connected to the immunological activities of colorectal cancer. The test results are reported as either "negative" or "positive" for the likely presence of CRC. The test is reported to have 94% accuracy in determining the "likely presence or absence of colorectal cancer." "BeScreened™-CRC is not a test for colorectal cancer diagnosis; it is a screening test that aides in the detection of colorectal cancer and is not intended to replace a colonoscopy" (BeScreened, 2023).

REVEAL Lung Nodule Characterization is a blood test that specifically aids in "characterizing indeterminate pulmonary nodules (4-30mm) in current smokers aged 25 years and older." The test results are based on three clinical factors and three blood proteins associated with lung cancer. "REVEAL Lung Nodule Characterization is a risk assessment tool, that is to be used only in conjunction with standard clinical assessments. The test is not intended as a screening or stand-alone diagnostic assay" (MagArray, 2023).

OVA1® and OVERA® are blood tests for ovarian cancer. OVA1® has FDA-clearance for testing ovarian cancer risk in women planning to have surgery for a pelvic mas. OVA1® is a first-generation multivariate index assay that measures five ovarian cancer-associated markers. "A negative OVA1 (MIA) result is accompanied by a 98% likelihood that the woman being tested is in fact disease-free." OVERA® has been FDA-cleared for people with a pelvic mass who are planned for surgery. OVERA® is a second-generation multivariate index assay that measures different markers and uses a more refined algorithm than OVA1® (ASPIRE, 2023).

Clinical Utility and Validity

Most biomarkers are not specific for tumors or organs, and their levels may rise in other diseases. The diagnostic value of a tumor marker will depend on the prevalence of the disease and on the specificity and sensitivity of the marker (Hottinger & Hormigo, 2011). The analytic and clinical validity as well as the clinical utility of each biomarker should be taken into account before its use for screening and or management of malignancies (Catharine M. Sturgeon et al., 2008). Establishing a biomarker's ability to associate with a given outcome of interest (diagnostic, prognostic, et al.) and ability to improve clinical outcomes and decision-making is critical (Febbo et al., 2011).

With respect to biomarker acquisition, growing evidence continues to support the utility of liquid biopsy. Compared to the "gold standard" tissue biopsy, serum can be obtained in a relatively non-invasive manner, without the need for surgery and the associated risks and recovery time. Further, serum is generally always available; tumor tissue, conversely, may not always be accessible or present in a clinically useful quantity (Pinzani et al., 2021).

Alpha-fetoprotein (AFP)

Alpha-fetoprotein (AFP) is a commonly assessed biomarker in cancer patients. AFP is a protein that is normally produced by the fetal yolk sac, and its concentration stabilizes at approximately < 10 µg/L shortly after birth (Schefer, Mattmann, & Joss, 1998). Many tissues produce this protein if they become malignant, and AFP is elevated in a variety of cancers, such as hepatocellular carcinomas. False positives may occur due to liver damage or a rare hereditary syndrome (Gilligan et al., 2010).

Alpha-fetoprotein can be fractionated into three different isoforms based on reactivity with Lens culinaris agglutinin (LCA), and the three types are as follows: L1 (no reactivity), L2 (low reactivity), L3 (high reactivity). AFP-L3 is theorized to associate with HCC because the dedifferentiation of HCC tissues correlates with the production of the enzyme that produces AFP-L3. This means that AFP-L3 may be closely related to cancer-specific events and are at least more specific to more malignant cancers (Wu et al., 2018).

A study by Santos Schraiber et al. (2016) assessed the ability to predict recurrence of hepatocellular carcinoma (HCC) after liver transplant using AFP. The authors analyzed 206 patients, and the recurrence frequency was found to be 15.5%. However, the authors' multivariate analysis found that the only risk factor for recurrence was an AFP level of >200 ng/mL, which was associated with a 3.32 times higher increase in the probably of HCC recurrence. The authors noted that recurrence was also associated with lower survival rate (Santos Schraiber et al., 2016).

Cheng et al. (2014) conducted a meta-analysis of fifteen studies (4465 patients) to evaluate the association between high pre-treatment serum AFP-L3% and overall survival (OS) and disease-free survival (DFS) in HCC patients. The authors found that high pre-treatment serum AFP-L3% implied poor OS (Hazard Ratio [HR]: 1.65, and DFS (HR: 1.80) of HCC. The authors found an association between pre-treatment serum AFP-L3% and OS and DFS in low AFP concentration HCC patients (HR: 1.96 and 2.53 respectively). The authors concluded that "high pre-treatment serum AFP-L3% levels indicated a poor prognosis for patients with HCC" (Cheng et al., 2014).

Park et al. (2017) compared the diagnostic values of AFP, AFP-L3, and PIVKA-II individually and in combination to find the best biomarker or biomarker panel. A total of 79 patients with newly diagnosed HCC and 77 control patients with liver cirrhosis were enrolled. When the three biomarkers were analyzed individually, AFP showed the largest area under the receiver-operating characteristic curve (AUC) (0.751). For combinations of the biomarkers, the AUC was highest (0.765) for PIVKA-II > 40 mAU/mL and AFP > 10 ng/mL. Adding AFP-L3 > 10% led to worse sensitivity and lower AUC. The authors concluded that "the diagnostic value of AFP was improved by combining it with PIVKA-II, but adding AFP-L3 did not contribute to the ability to distinguish between HCC and non-HCC liver cirrhosis" and that "AFP showed the best diagnostic performance as a single biomarker for HCC" (Park et al., 2017).

Ryu et al. (2017) investigated the prognostic implications of the expression patterns of three tumor markers, alpha-fetoprotein (AFP), the Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP). The study included 1182 consecutive patients that underwent hepatic resection and surgical microwave ablation for HCC. This study analyzed 475 patients within the Milan criteria and Child-Pugh class A. Cumulative overall survival (OS) and disease-free survival (DFS) rates were analyzed relative to the number of positive tumor markers. OS and DFS at five years postoperatively were

85.3 and 44.2% in triple-negative patients, 79.4 and 48.0% in single-positive patients, 56.2 and 32.9% in double-positive patients, and 61.7 and 35.7% in triple-positive patients. OS in triple-negative or single-positive patients was 85.3%, and that in all double- or triple-positive patients was 58.0%; DFS at five years postoperatively in these two groups was 45.9 and 34.0%, respectively. The authors concluded that "both double- and triple-positive tumor markers are associated with early recurrence and poor survival in HCC patients within the Milan criteria and Child-Pugh class A" (Ryu et al., 2017).

Caviglia et al. (2016) conducted a study evaluating AFP, AFP-L3, and DCP as detection tools for HCC. A total of 98 patients were enrolled (44 without HCC, 54 with), and the FDA-approved uTASWako was used to measure these biomarkers. AFP-L3 had an area under the curve of 0.867, a sensitivity of 0.849, a specificity of 0.886, a negative predictive value of 0.830, and a positive predictive value of 0.900. The combination of all three biomarkers had an accuracy of 87.6%. The overall accuracy of uTASWako was 84.5%. The authors concluded that the uTASWako had a "high analytical performance" and that the biomarker combination was superior to any of them alone (Caviglia et al., 2016).

Beta-human chorionic gonadotropin (beta-HCG)

beta-HCG is the beta subunit of the normal hCG hormone produced during pregnancy. Some malignancies express the gene for the beta subunit of hCG, thereby producing this protein outside of pregnancy (Goff, 2016; Harvey, 2019). The beta subunit is responsible for providing the biological and immunological specificity to each hormone (Marcillac et al., 1992) This biomarker is typically associated with aggressive disease in nontrophoblastic tumors. This biomarker may be seen in ovarian cancers, testicular cancers, and more (Hotakainen et al., 2002).

Li et al. (2018) evaluated beta-hCG as a marker for colorectal cancer (CRC). In total, 50 patients out of 136 patients expressed beta-hCG at the "invasive front." The authors found that higher expression of beta-hCG to be associated with worse prognosis than those with low beta-hCG expression and that beta-hCG "promoted the migration and invasion of CRC in vitro and in vivo but had no effect on the proliferation of tumor cells." A correlation was also found between beta-HCG expression level and tumor invasion in early-stage CRC patients (Li et al., 2018).

Beta-2 microglobulin (B2M)

B2M is the light chain component of the MHC-1 molecule and is present in most cells of the body (Berrebi et al., 2009). This protein may aggregate and eventually form insoluble amyloid fibrils, which cause numerous conditions such as bone and joint damage (Katou et al., 2002; Marcinko, Dong, LeBlanc, Daborowski, & Vachet, 2017). Elevated serum levels of B2M have been associated with cancers such as multiple myeloma or chronic leukocytic leukemia (Berrebi et al., 2009)

Seo et al. (2016) examined the prognostic value of B2M for diffuse large B-cell lymphoma. A total of 833 patients at a ≥2.5 mg/L cutoff were analyzed, and both five-year survival and overall survival rates were found to be significantly worse in patients with elevated B2M (290 patients or 34.8%). The elevated B2M cohort was calculated to have a 41% five-year survival rate and a 49.2% overall survival rate, compared to 76.1% five-year survival and 83.8% overall survival for the remaining 543 patients (Seo et al., 2016).

Calcitonin

Serum calcitonin is the primary tumor marker for medullary thyroid carcinoma (MTC). MTC is a neuroendocrine tumor of the parafollicular or C cells of the thyroid gland, and production of calcitonin is a signifying characteristic of this tumor. The concentration of calcitonin tends to correlate with tumor mass (Tuttle, 2023). However, the ATA has noted this biomarker to have significant uncertainties (Haugen et al., 2016; Wells et al., 2015).

Tormey et al. (2017) evaluated measurement of serum calcitonin in patients presenting with thyroid nodules. A total of 44 patients were evaluated, and 33 of the patients did not have a "detectable serum calcitonin," noting that three patients had an initially elevated serum concentration that became undetectable. The authors also note that out of the 2070 patients in their sample, only seven cases of medullary thyroid cancer (MTC) were diagnosed. The authors recommended not screening routinely for MTC (Tormey et al., 2017).

Cancer antigens (CA)

Cancer antigens (CA) refer to any substance produced by the body in response to a tumor. Various cancer antigens have been proposed as biomarkers for numerous types of cancer, such as CA 19-9, CA 125, and CA 15-3. CA 19-9 (also called carbohydrate antigen) refers to a specific antibody that binds a sialyl compound produced by cancer tissue (Sialyl Lewis A). CA 19-9 is elevated in several different types of cancer, such as adenocarcinomas or colorectal cancer (Magnani, 2004). CA 125 is a glycoprotein produced in fetal tissue as well as mesothelial cells in adults (Isaksson et al., 2017). Its function is thought to assist with cell adhesion, metastasis, and immunosuppression (Dorigo & Berek, 2011).

Kim et al. (2017) performed a study assessing the association of serum CA 19-9 and CEA with colorectal neoplasia. A total of 124509 measurements of serum CEA level and 115833 measurements of serum CA 19-9 were taken. All subjects were asymptomatic and underwent a colonoscopy. Elevated serum levels of CEA were found to be associated with any adenoma. Elevated CA 19-9 was found to be associated with high-risk or advanced adenoma, CRC, and advanced colorectal neoplasia (Kim et al., 2017).

A study was performed by Feng et al. (2017) that focused on the diagnostic and prognostic value of CEA, CA 19-9, AFP, and CA125 for early gastric cancer. The authors evaluated 587 patients, and the positive rate for all markers combined was 10.4%. CEA's positive rate was 4.3%, CA 19-9's was 4.8%, AFP's was 1.5%, and CA125's was 1.9%. The authors noted that elevated CEA was correlated with lymph node metastasis and concluded that CEA was an independent risk factor for poor prognosis of early gastric cancer (Feng et al., 2017).

Lucarelli et al. (2014) evaluated CA 15-3, CA125, and B2M as biomarkers for renal cell carcinoma (RCC). A total of 332 patients undergoing nephrectomy for RCC were analyzed. The authors found that 35.2% (117/332) of patients had abnormal levels of CA 15-3, 9.6% (32/332) had abnormal levels of CA125, and 30.4% (101/332) had abnormal B2M. Cancer specific survival (CSS) rates significantly decreased for high levels of any of the three biomarkers, and at a multivariate analysis found high levels of CA 15-3 to be an independent adverse prognostic risk factor for CSS (Lucarelli et al., 2014).

Chen et al. (2018) analyzed four serum tumor markers in patients with ovarian tumors. HE4, CA-125, CA19-9, and CEA were all studied. The authors evaluated 386 healthy controls, 262 patients with benign ovarian tumors, and 196 patients with malignant ovarian tumors. The authors found that the serum marker levels were significantly higher in patients with malignant tumors than the two other groups. HE4 was found to have a high specificity (96.56%) in malignant tumors. HE4, CA125, CA19-9, and CEA had sensitivities of 63.78%, 62.75%, 35.71%, and 38.78%, respectively. HE4 and CA125 combined was found to have the highest diagnostic sensitivity at 80.10%, as well as a specificity of 69.08%. Although adding markers to the HE4-CA125 combination increased diagnostic sensitivity (to 88.52%), this difference was not considered significant (Chen et al., 2018).

Isaksson et al. (2017) performed a study of tumor markers' association with resectable lung adenocarcinomas. The study evaluated blood samples from 107 patients with stages I-III lung adenocarcinoma and examined the following markers: CEA, CA 19-9, CA 125, human epididymis protein 4 (HE4), and neuron-specific enolase (NSE). When the authors calculated the disease-free survival rate, CA 19-9 and CA 125 were found to be significantly associated with recurrent disease with a combined hazard ratio of 2.8. The authors stated that "high pre-operative serum CA 19–9 and/or CA 125 might indicate an increased incidence of recurrent disease in resectable lung adenocarcinomas" (Isaksson et al., 2017).

Bind et al. (2021) evaluated the diagnostic ability of CA19-9 and CA 125 for gallbladder cancers. A total of 118 patients were included, 91 benign cases and 27 malignant. The mean value of CA19-9 was found to be 12.86 U/mL in benign cases and 625.35 U/mL in malignant cases. For CA 125, the mean value for benign cases was found to 17.98 U/mL, and for malignant cases, 239.63 U/mL. The authors examined a theoretical diagnostic cut-off value of 252.31 U/mL for CA19-9 and 92.19 U/mL for CA 125. At this cutoff, sensitivity and specificity for CA19-9 was 100% and 98.9% respectively, and for CA 125, 100% and 94.5%. The authors concluded that "…both serum CA 19-9 and serum CA 125 may act as a good adjunct for diagnosis of cases of carcinoma gallbladder along with imaging studies. However, changes in CA19-9 are more significant than CA 125" (Bind et al., 2021).

Carcinoembryonic antigen (CEA)

CEA is a protein normally produced by fetal tissue, and as with AFP, stabilizes soon after birth. CEA is often elevated in malignancies such as breast or pancreatic cancer, although other conditions such as liver damage or cigarette smoking may affect CEA levels as well (F. Ueland, 2017; F. R. Ueland, Li, Andrew John, 2020). The gene encoding CEA encompasses certain genes encoding for cell adhesion, as well as MHC antigens (Duffy, 2001).

Chromogranin A (CgA)

Chromogranins are proteins contained in neurosecretory vesicles of NET cells and are typically elevated in neuroendocrine neoplasms. CgA is the most sensitive of the three chromogranins, and as such as the primary marker used to evaluate neoplasms. However, this biomarker is highly variable (Strosberg, 2017, 2019).

A meta-analysis performed by Yang et al. (2015) assessed the association of CgA with neuroendocrine tumors. The analyses included 13 studies totaling 1260 patients (967 healthy controls), and the pooled sensitivity was found to be 0.73. The pooled specificity was found to be 0.95. However, the study stressed that further research needs to be undertaken (Yang et al., 2015). Another study by Tian et al. (2016) found that although median CgA levels were significantly higher than healthy controls (93.8 ng/mL compared to 37.1 ng/mL), only a weak correlation was found between changes in serum CgA levels and clinical regimen. The CgA cutoff value for this study was 46.2 ng/mL, which led to a sensitivity of 78.8% and specificity of 73.8% (Tian et al., 2016).

Lactate Dehydrogenase (LDH)

Lactate Dehydrogenase (LDH) is an enzyme that catalyzes the interconversion between lactate and pyruvate. LDH is often found to be upregulated in tumors, and a key feature of cancer sites is the accumulation of lactate or lactic acid. This is thought to be caused by increased glycolysis and this increase in lactate causes an elevated concentration of LDH (Pucino et al., 2017). Increased LDH is found in several different cancers, such as B-cell lymphomas and osteosarcomas (NCCN, 2023t).

Liu et al. (2016) performed a study evaluating the OS rates of an extremely high concentration of LDH (>1000 IU/L, considered by the study to be four times the upper normal limit). A total of 311 patients with >1000 U/L were examined, and the OS rate of this cohort was 1.7 months with 163 perishing within two months. However, 51 patients' LDH decreased to normal following chemotherapy, and the OS rate of this group was 22.6 months. The cohort who survived at two months but did not see their LDH decrease had an OS rate of four months. There was no positive association found between OS and type of cancer, although there were different OS rates for patients at different stages of lymphoma (Liu et al., 2016).

Inhibins

The primary function of inhibins is to inhibit hormones such as follicle stimulating hormone. However, since this protein is restricted to ovarian granulosa cells in women, unusual levels of inhibins may signal tumors in this region (Walentowicz et al., 2014). This marker exists as two different isoforms, inhibin A and B. Either form can be measured, although an active tumor may over-secrete one or both forms (Gershenson, 2023). Inhibin B is generally considered to be more accurate than inhibin A, with sensitivities ranging from 0.88 to 1.00 whereas inhibin A's sensitivity ranges from 0.67-0.77. However, inhibin B has limitations of its own such as fluctuations with the menstrual cycle (Farkkila et al., 2015).

Farkkila et al. (2015) evaluated anti-Müllerian hormone (AMH) and inhibin B in the context of ovarian adult-type granulosa cell tumors (AGCTs). The study included 560 samples taken from 123 patients, and both markers were significantly elevated in AGCTs. The area under the curve for inhibin B was 0.94, but measurement of both markers was noted to be a better method than measuring either marker individually (Farkkila et al., 2015).

Urokinase plasminogen activator

Urokinase plasminogen activator (uPA) is a serine protease with an important role in cancer invasion and metastases (Stephens, Brunner, Janicke, & Schmitt, 1998). When bound to its receptor (uPAR), uPA converts plasminogen into plasmin and mediates degradation of the extracellular matrix during tumor cell invasion. High levels have been associated with shorter survival in women with breast cancer (Chappuis et al., 2001; Foekens et al., 2000; Malmstrom et al., 2001; Stephens et al., 1998). ASCO guidelines include the option for using uPA and PAI-1 to guide decisions on adjuvant systemic therapy for patients with node-negative, hormone-positive/HER2-negative disease, but not for patients with HER2-positive or triple-negative disease (L. N. Harris et al., 2016; Theodoros & Bergh, 2023).

Serum free light chains

Light chains are proteins produced by plasma cells that, along with heavy chains, collectively make up an immunoglobulin macromolecule. There are a total of 5 heavy chain protein classes (IgG, IgE, IgA, IgD, and IgM), and 2 light chain protein classes (kappa and lambda). Healthy plasma cells produce polyclonal immunoglobulins that are capable of binding to antigens and inducing an immune response; unhealthy plasma cells produce monoclonal immunoglobulins that do not effectively engage antigens (Kyrtsonis MC, 2012). In the case of certain plasma cell disorders, an abundance of monoclonal immunoglobulin or free light chains (kappa and/or lambda), may accumulate in the serum and serve as useful diagnostic markers.

Multiple myeloma

Multiple myeloma is an uncontrolled growth of plasma cells (ACS, 2018a). In most cases, the cancerous clonal cells secrete an intact monoclonal immunoglobulin, and the gold standard for diagnosis is serum protein electrophoresis and immunofixation (Tosi et al., 2013). Less commonly, however, myeloma clones will secrete only light chains; in these instances, a serum free light chain assay can be employed to quantify the ratio of kappa and lambda chains in the serum. It has been demonstrated that in healthy individuals, the kappa/lambda ratio in the serum is approximately 0.58 (Katzmann et al., 2002). In the case of plasma cell neoplasms, free light chains are overproduced and the kidneys are unable to completely clear them, resulting in accumulation in the serum and a change in the kappa/lambda ratio. This ratio is often used to aid in the diagnosis, prognosis, and monitoring of plasma cell disorders (Tosi et al., 2013).

Waldenström's Macroglobulinemia (WM) is a type of cancer that is similar to multiple myeloma and non-Hodgkin lymphoma. WM cells are called “lymphoplasmacytoid” because they have features of both plasma cells and lymphocytes (ACS, 2018b). WM cells are distinguished by the production of immunoglobulin M (IgM) serum monoclonal protein, also referred to as a “macroglobulin” (Cautha et al., 2022). While serum IgM level is useful for diagnostic purposes, it does not correlate with prognosis. The addition of a serum free light chain assay to the care of patients with suspected Waldenström's Macroglobulinemia has been shown to improve overall care, as it may help differentiate patients with another, potentially benign disorder called monoclonal gammopathy of undetermined significance (MGUS), as well as influence prognosis (Moreau AS, 2006).

Castleman disease represents a group of B-cell lymphoproliferative disorders characterized by distinct pathogenesis and clinical outcomes (Oyaert et al., 2014; D. Wu et al., 2018). Patients with suspected

Castleman disease have been reported to present with abnormal levels or kappa or lambda light chains, making the serum free light chain assay a potentially useful tool in the management of this disease (Oyaert et al., 2014; D. Wu et al., 2018). Utilization of a serum free light chain assay has been shown to be clinically useful in the workup of Castleman disease, though an important caveat is that changes in the absolute values of both kappa and lambda free light chain in the serum can occur with preservation of a ratio within the normal reference range (Stankowski-Drengler et al., 2010); hence, both the free light chain ratio as well as the absolute values of each light chain protein should be considered.

Immunoglobulin light chain amyloidosis is a disorder that results from the accumulation of amyloid fibrils due to the production of fragments of monoclonal light chains (Dispenzieri, 2023; Merlini et al., 2013). As amyloid fibrils continue to accumulate, they begin to interfere with the biological function of various organs, eventually resulting in organ damage and potentially organ failure. Due to the involvement of light chains in the pathogenesis of amyloidosis, serum free light chain measurement may hold diagnostic and prognostic value, and be a viable response marker following therapy (Akar et al., 2005; Bhole et al., 2014; Kumar et al., 2010).

Importantly, Bhole and colleagues highlighted key challenges with serum free light chain assays that include but are not limited to over or under-estimation of the monoclonal protein, and performance differences between available tests. Therefore, despite the demonstrated utility of these assays, clinicians should be aware of their limitations.

Proteomics

Proteomics is a qualitative and quantitative assessment of the protein constituents in a given biological sample. This is typically performed with modification of polyacrylamide gel electrophoresis (PAGE) or matrix-assisted laser desorption/ionization (MALDI). However, this method is still under investigation (Raby, 2023).

Proteomic analyses have been performed in cancer patients to assess unusual levels of protein regulation. A study by Chen et al. (2017) evaluated the proteomes of patients with CRC and healthy controls. Chen et al found 36 proteins that were upregulated in cancer patients as well as 22 proteins that were downregulated compared to healthy controls. The proteins that were upregulated tended to be processes that regulated the "pretumorigenic microenvironment for metastasis" and the downregulated proteins tended to be ones that controlled tumor growth and cell survival (Chen et al., 2017).

Qin et al. (2020) performed a "serological proteome analysis" to explore the association between an identified protein marker and gastric cancer (GC). Proteomic analysis was used to identify the protein marker of interest, an autoantibody called "anti-GRP78" (along with its corresponding antigen, the 78-kDa glucose-regulated protein [GRP78]). Two cohorts were included, a test group of 266 patients (133 GC patients, 133 controls) and a validation group of 600 patients (300 GC, 300 control). The authors found that the levels of anti-GRP78 was higher in both cohorts. The receiver operating characteristic (ROC) curve analysis found similar values for both groups to identify GC patients among control patients. The area under curve (AUC) ranged from 0.676 to 0.773 in the test group and 0.645 to 0.707 in the validation group. The authors noted this marker's potential use as a diagnostic marker (Qin et al., 2020).

IV. Guidelines and Recommendations

National Academy of Clinical Biochemistry (NACB) now known as the American Association for Clinical Chemistry (AACC)

The National Academy of Clinical Biochemistry published practice Guidelines for the use of major tumor markers for Liver, Bladder, Cervical, and Gastric Cancers (Sturgeon et al., 2010).

The NACB recommends use of AFP measurements when managing hepatocellular carcinoma (HCC). For screening, the NACB recommends AFP be measured at 6-month intervals in patients at high risk of HCC, noting concentrations above 20 µg/L should "prompt further investigation even if an ultrasound is negative." Sustained increases of serum AFP may be used with ultrasound to inform detection and management, and AFP concentrations may provide prognostic information in untreated patients. Monitoring of disease should include measurement of AFP. However, other liver biomarkers such as Glypican-3 cannot be recommended at this time as further research is needed. (Sturgeon et al., 2010)

The NACB did not recommend any biomarkers for the management of bladder cancer (such as NMP22, UroVysion, etc), stating that further research is required to assess their utility. The NACB did not recommend any biomarkers for screening, monitoring, prognosis, or diagnosis of cervical cancer. Pretreatment measurements of squamous cell carcinoma antigen (SCC) were acknowledged to provide information, but their routine use could not be recommended. The NACB did not recommend any biomarkers for screening, diagnosis or prognosis of gastric cancer. Routine measurement of CEA or CA 19-9 was also not recommended (Sturgeon et al., 2010).

The NACB also published guidelines on use of major tumor markers for Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers (C. M. Sturgeon et al., 2008). For testicular cancer, the NACB stated that pretreatment determination of AFP, lactate dehydrogenase (LDH), and human chorionic gonadotropin (hCG) was "mandatory" if testicular cancer was suspected or if risk stratification and staging was done. These three biomarkers were also recommended for monitoring. NACB also notes that measurement of the hCGβ component is essential when measuring hCG. For prostate cancer, PSA assessment is required during all stages of the disease. NACB recommends against age-specific intervals for PSA. However, the NACB also did not make any recommendations on PSA screening for prostate cancer (C.M. Sturgeon et al., 2008).

For colorectal cancer (CRC), carcinoembryonic antigen (CEA) measurement is recommended every 3 months in stage II or III CRC if "patient is a candidate for surgery or systemic therapy of metastatic disease". Pre-operative CEA measurements may be used in conjunction with other factors to plan surgery. Regular CEA measurements should be done in patients with advanced CRC that are undergoing systemic therapy. CEA is not recommended for screening in healthy individuals. Routine measurement of other biomarkers such as CA 19-9, TIMP-1, or CA 242 is not recommended, for prognosis or predicting response to treatment. The NACB recommends individuals older than 50 to be screened for CRC. Fecal DNA is also recommended for CRC screening as joint guidelines from other societies such as the

American Cancer Society have recommended its use. Finally, the NACB supports guidelines such as the NCCN and AGA regarding genetic testing for CRC (C. M. Sturgeon et al., 2008).

For breast cancer, the NACB states estrogen receptor (ER) and progesterone receptor (PR) measurement should be done in all patients with breast cancer. HER-2 should be measured in all patients with invasive breast cancer. urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) may be used to identify "lymph node–negative breast cancer patients who do not need or are unlikely to benefit from adjuvant chemotherapy." CA 15-3, CEA, and BR 27.29 should not be routinely used for early detection in asymptomatic patients with diagnosed breast cancer. BRCA1 and BRCA2 mutation testing may be used to identify women at high risk of developing breast or ovarian cancer. OncoType DX may be used to predict recurrence in "lymph node–negative, ER-positive patients receiving adjuvant tamoxifen". However, microarray-based gene signatures should be routinely used for predicting patient outcome (C. M. Sturgeon et al., 2008).

For ovarian cancer, CA125 is not recommended for screening asymptomatic women, but is recommended (with transvaginal ultrasound) for early detection of ovarian cancer in women with hereditary syndromes. CA125 is also recommended as distinguishing benign from malignant masses and may be used to monitor response to chemotherapeutic response. Measurement of CA125 during follow-up visits is recommended if initial values were increased. CA125 measurement is also recommended during primary therapy. Other biomarkers such as inhibin and hCG cannot be recommended at this time (C. M. Sturgeon et al., 2008).

American Society of Clinical Oncology (ASCO)

The ASCO released Clinical Practice Guideline on Uses of Serum Tumor Markers (STMs) in Adult Males With Germ Cell Tumors (GCTs) in 2010 (Gilligan et al., 2010). ASCO recommends against any STMs to screen for GCTs. ASCO recommends assessment of serum AFP and hCG before orchiectomy to establish a diagnosis and baseline levels but recommends against its use to decide whether to perform an orchiectomy. The society also recommends against using these biomarkers to "guide treatment of patients with CUP and indeterminate histology". However, substantially elevated serum AFP and/or hCG may be considered sufficient for a diagnosis in unusual cases such as patients presenting with a retroperitoneal, or anterior mediastinal primary tumor. Their recommendations also include measuring serum AFP, hCG, and LDH for "all patients with testicular nonseminomatous germ cell tumors (NSGCTs) shortly after orchiectomy and before any subsequent treatment", "before chemotherapy begins for those with mediastinal or retroperitoneal NSGCTs to stratify risk and select treatment", and "immediately prior to chemotherapy for stage II/III testicular NSGC" (Gilligan et al., 2010).

The ASCO recommends measuring AFP and hCG before retroperitoneal lymph node dissection in patients with stage 1 or II NSGCT. ASCO recommends measuring serum AFP and hCG at the start of each chemotherapy cycle and when chemotherapy concludes. These biomarkers are also recommended to be measured during surveillance after "definitive therapy for NGSCT" and that surveillance should continue for 10 years after therapy concludes (Gilligan et al., 2010).

The ASCO recommends measuring "postorchiectomy serum concentrations of hCG and/or LDH for patients with testicular pure seminoma and preorchiectomy elevations" but recommends against using these concentrations for staging or prognosis. No markers are recommended to guide treatment decisions, monitor response, or progression for seminomas. However, serum hCG and AFP should be measured when treatment concludes and during post-treatment surveillance. ASCO recommends these intervals: every two to four months in the first year, every three to four months in the second year, every four to six months in the third and fourth years, and annually thereafter. Surveillance should last for at least 10 years after therapy concludes (Gilligan et al., 2010).

The ASCO also published joint guidelines with the **American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology** regarding biomarkers for colorectal cancer. In it, they do not mention any serum tumor markers for testing (Sepulveda et al., 2017).

The ASCO also released guidelines on use of biomarkers to inform treatment decisions on systemic therapy for women with metastatic breast cancer. "Patients with accessible, newly diagnosed metastases from primary breast cancer should be offered biopsy for confirmation of disease process and testing of ER, PR, and HER2 status. With discordance of results between primary and metastatic tissues, the panel consensus is to preferentially use the ER, PR, and HER2 status from the metastasis to direct therapy if supported by the clinical scenario and the patient's goals for care." Decisions on changing to a new drug or regimen, initiating, or discontinuing treatment should be based on the patient's goals for care and clinical evaluation and judgment of disease progression or response. There is no evidence at this time that changing therapy solely based on tissue or circulating biomarker results beyond ER, PR, and HER2 improves health outcomes, quality of life, or cost-effectiveness. To date, clinical utility has not been demonstrated for any additional biomarkers. "CEA, CA 15-3, and CA 27.29 may be used as adjunctive assessments to contribute to decisions regarding therapy for metastatic breast cancer. Data are insufficient to recommend use of CEA, CA 15-3, and CA 27.29 alone for monitoring response to treatment" (Van Poznak et al., 2015).

The ASCO released a focused update for women with early-stage invasive breast cancer, which is as follows (Krop et al., 2017).

- If a patient has ER/PgR-positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay may be used in those with high clinical risk, but not low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy (Krop et al., 2017).
- If a patient has ER/PgR-positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay may be used in patients with 1-3 positive nodes and at high clinical risk, but not for patients at low clinical risk (Krop et al., 2017).
- If a patient has HER2-positive breast cancer or ER/PgR negative and HER2-negative breast cancer (triple negative the clinician should not use the MammaPrint assay (Krop et al., 2017).

The following recommendations were unchanged from the 2016 version:

- If a patient has ER/PgR-positive, HER2-negative (node-positive or node-negative) breast cancer, the clinician should not use tumor-infiltrating lymphocytes (TILs), the five-protein assay (Mammostrat), and the immunohistochemistry 4 (IHC4) assay, to guide decisions on adjuvant systemic therapy (Krop et al., 2017).
- If a patient has ER/PgR-positive, HER2-negative (node-positive) breast cancer, the clinician should not use the Breast Cancer Index, the PAM50-ROR, the 12-gene risk score, or the 21-gene RS, (EndoPredict), to guide decisions on adjuvant systemic therapy (Krop et al., 2017).
- If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use urokinase plasminogen activator (uPA), plasminogen activator inhibitor type 1 (PAI-1), the Breast Cancer Index, the PAM50 risk of recurrence (ROR) score, the 12-gene risk score (EndoPredict), and the 21-gene recurrence score (Oncotype DX) to guide decisions on adjuvant systemic therapy. If the patient has had 5 years of endocrine therapy without evidence of recurrence, the clinician should not use multiparameter gene expression or protein assays (Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, or IHC4) to guide decisions on extended endocrine therapy (Krop et al., 2017).
- If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use uPA, IHC4, the 12-gene risk score (EndoPredict), PAI-1, the 21-gene RS (Oncotype DX), the five-protein assay (Mammostrat), the Breast Cancer Index or TILs to guide decisions on adjuvant systemic therapy. The clinician should not use circulating tumor cells (CTCs) to guide decisions on adjuvant systemic therapy (Krop et al., 2017).
- The clinician should not use *CYP2D6* polymorphisms, p27 expression, Ki-67 labeling index, microtubule-associated protein (MAP)-Tau mRNA expression or mRNA expression, HER1/epidermal growth factor receptor (EGFR) expression, TOP2A gene amplification or TOP2A protein expression, HER2 and TOP2A gene coamplification; CEP17 duplication; or TIMP-1, FOXP3, or p53 protein expression (all by IHC) to guide adjuvant endocrine therapy selection. If a patient has HER2-positive breast cancer, the clinician should not use PTEN or soluble HER2 levels to guide adjuvant therapy selection (Krop et al., 2017).
- If a patient has HER2-positive breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy (Krop et al., 2017).
- If a patient has TN breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy (Krop et al., 2017).

The ASCO released a provisional clinical opinion on evaluating susceptibility to pancreatic cancer, stating that "there are currently no proven biomarkers using noninvasively obtained biospecimens (eg, blood, urine, stool) for early detection of pancreatic cancer in asymptomatic individuals." ASCO states that further validation of biomarkers is needed (Stoffel et al., 2018).

The ASCO also released a guideline on treatment of malignant pleural mesothelioma, stating that calretinin, keratins 5 and 6, and nuclear WT-1 are expected to be positive while CEA, EPCAM, Claudin 4, and TTF-1 should be negative. Non-tissue based biomarkers are currently not recommended due to their unvalidated statistical accuracy (Kindler et al., 2018).

The ASCO remarks that "If a patient has ER/PgR-positive, HER2-negative **(**node-negative**)** breast cancer, the clinician may use urokinase plasminogen activator and plasminogen activator inhibitor type 1 to guide decisions on adjuvant systemic therapy." The guidelines also recommend against using IHC4 to guide decisions on adjuvant systemic therapy (Lyndsay N. Harris et al., 2016).

The ASCO released an update on ER/PgR testing for breast cancer. In it, they continue to recommend ER testing for predicting success of endocrine therapy. They remark that similar principles apply to PgR testing, which provides prognostic information for ER+ cancer. ASCO also recommends testing ER for patients with ductal carcinoma in situ (DCIS) to determine benefit of endocrine therapies (Allison et al., 2020).

American College of Chest Physicians (ACCP)

In 2013, the ACCP published evidence-based clinical practice guidelines for diagnosis and management of lung cancer. The guidelines did not mention proteomic markers as a potential diagnostic or screening tool (Detterbeck et al., 2013).

National Institute for Health and Clinical Excellence (NICE)

The National Institute for Health and Clinical Excellence (NICE) issued guidelines in 2011 on the recognition and initial management of ovarian cancer. It stated that the routine use of CA-125 is recommended; the data on other serum markers is not substantial enough to recommend their use. It included the following recommendations:

1. Measure serum CA-125 in primary care in women with symptoms that suggest ovarian cancer.
2. If serum CA-125 is 35 IU/mL or greater, arrange an ultrasound scan of the abdomen and pelvis.
3. If the ultrasound suggests ovarian cancer, refer the woman urgently for further investigation.
4. For any woman who has normal serum CA-125 (less than 35 IU/mL), or CA-125 of 35 IU/mL or greater but a normal ultrasound: 1) assess her carefully for other clinical causes of her symptoms and investigate if appropriate; 2) if no other clinical cause is apparent, advise.
5. Calculate a risk of malignancy index I (RMI I) score (after performing an ultrasound). (The RMI 1 combines CA-125, menopausal status and the ultrasound score).

The NICE guidelines also suggested to measure AFP, beta-hCG and serum CA125 in women under 40 with suspected ovarian cancer (NICE, 2011).

NICE also recommends using a serum free-light chain assay to "confirm the presence of a paraprotein indicating possible myeloma or monoclonal gammopathy of undetermined significance (MGUS)." Serum immunofixation is recommended if serum protein electrophoresis is abnormal. Finally, serum free light-chain ratio is recommended for prognosis (NICE, 2018).

Serum Tumor Markers for Malignancies, continued

National Comprehensive Cancer Network (NCCN)

Marker	Recommendation	Source
AFP	Hepatocellular workup (HCC). AFP for surveillance and screening is optional. AFP testing (every 6 months) for patients with established risk factors for HCC.	NCCN Hepatocellular Carcinoma Version 1.2023 (NCCN, 2023k)
AFP	Respectable HCC. Surveillance imaging and AFP should continue for at least 5 years and thereafter screening is dependent on HCC risk factors.	
AFP	Consider for intrahepatic cholangiocarcinoma workup	NCCN Hepatocellular Carcinoma Version 1.2023 (NCCN, 2023k)
AFP	Individuals younger than 35 years with a pelvic mass should have AFP levels measured to assess for germ cell tumors and to rule out pregnancy. Patients achieving a complete clinical response after chemotherapy for germ cell tumors should be observed clinically every 2 to 4 months with AFP and beta-HCG levels (if initially elevated) for 2 years.	NCCN Ovarian Cancer Version 1.2023 (NCCN, 2023x)
AFP	Thymomas and thymic carcinoma initial evaluation to rule our germ cell tumors.	NCCN Thymomas and Thymic Carcinomas Version 1.2023 (NCCN, 2023ai)
AFP	Both seminoma and nonseminoma, serum prognostic factor and contribute to diagnosis and staging. Also indicated for "suspicious testicular mass" workup	NCCN Testicular Cancer Version 1.2023 (NCCN, 2023ah)
AFP	Workup for occult primary mass of the liver, mediastinum, or retroperitoneum	NCCN Occult Primary Version 3.2023 (NCCN, 2023w)
Alkaline Phosphatase (ALP)	Should be assessed prior to treatment for bone cancers	NCCN Bone cancers Version 3.2023 (NCCN, 2023b)

Serum Tumor Markers for Malignancies, continued

Alkaline Phosphatase (ALP)	Part of the initial diagnostic workup for systemic light chain amyloidosis.		NCCN Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae)
Beta-HCG			NCCN Testicular Cancer Version 1.2023 (NCCN, 2023ah)
Beta-HCG	May be clinically indicated in the workup and management of epithelial ovarian, fallopian tube, primary peritoneal, and less common ovarian cancers.		NCCN Ovarian Cancer Version 1.2023 (NCCN, 2023x)
Beta-HCG	Workup for occult primary mass of the mediastinum or retroperitoneum		NCCN Occult Primary Version 1.2022 (NCCN, 2021f)
Beta-2 microglobulin (B2M)	Multiple myeloma initial workup and follow-up/surveillance		NCCN Multiple Myeloma Version 3.2023 (NCCN, 2023p)
Beta-2 microglobulin (B2M)	Workup for: follicular lymphoma (grades 1-2), mantle cell lymphoma, diffuse B-cell Lymphoma (useful in selected cases), AIDS-related B-cell lymphoma (useful in selected cases), lymphoblastic lymphoma workup (useful in selected cases), Castleman's disease		NCCN B-cell Lymphomas Version 2.2023 (NCCN, 2023a)
Beta-2 microglobulin (B2M)	Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma essential workup, prognostic factor		NCCN Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma Version 1.2023 (NCCN, 2023al)
Beta-2 microglobulin (B2M)	Workup and prognostic information ("useful under certain circumstances")		Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae)
Beta-2 microglobulin (B2M)	Initial diagnostic Workup		Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae)
BNP/NT-BNP	Initial diagnostic Workup (may be considered)		Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae)

Serum Tumor Markers for Malignancies, continued

Cancer Antigen 15-3 and 27.29		Cancer Antigen 15-3 and 27.29 as part of findings for identification of disease progression. An isolated increase in tumor marker should rarely be used as the only indicator of disease progression as these two biomarkers may also increase if metastatic disease is responding to treatment.	NCCN Breast Cancer Version 3.2022 (NCCN, 2022c)
Cancer Antigen 19-9		Pancreatic adenocarcinoma pre-op workup, post treatment, and surveillance, differential diagnosis, screening, staging, determining resectability, et al. Levels may be elevated up to 2 years before pancreatic CA diagnosis.	NCCN Pancreatic Adenocarcinoma Version 2.2022 (NCCN, 2023y)
Cancer Antigen 19-9	Workup and surveillance for small bowel adenocarcinoma		NCCN Small Bowel Adenocarcinoma Version 1.2022 (NCCN, 2022v)
Cancer Antigen 19-9		Gallbladder cancer initial workup (on findings such as jaundice or a mass found on imaging) and surveillance. CA 19-9 is a baseline test and should not be done to confirm diagnosis. Consider baseline CA 19-9 after biliary decompression. Intrahepatic (such as isolated intrahepatic mass) and extrahepatic cholangiocarcinoma workup. CA 19-9 is a baseline test and should not be done to confirm diagnosis	NCCN Hepatocellular Carcinoma Version 1.2022 (NCCN, 2023k)
Cancer Antigen 19-9		May be clinically indicated in the workup and management of epithelial ovarian, fallopian tube, primary peritoneal, and less common ovarian cancers	NCCN Ovarian Cancer Version 1.2023 (NCCN, 2023x)

Serum Tumor Markers for Malignancies, continued

CA-125		May be clinically indicated in the workup and management of epithelial ovarian, fallopian tube, primary peritoneal, and less common ovarian cancers	NCCN Ovarian Cancer Version 1.2023 (NCCN, 2023x)
CA-125		*BRCA* mutation-positive individuals – for those individuals who have not elected RRSO or TVUS serum CA-125 screening may be considered at the clinician's discretion starting age 30-35 years	NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 3.2023 (NCCN, 2023h)
CA-125		Endometrial carcinoma: Consider CA-125 for suspected extrauterine disease, serous carcinoma, clear cell carcinoma, carcinosarcoma, undifferentiated/dedifferentiated carcinoma; surveillance if initially elevated	NCCN Uterine Neoplasms Version 1.2023 (NCCN, 2023ak)
CA-125		Occult Primary – localized adenocarcinoma or carcinoma not otherwise specified workup for individuals in those with a uterus and/or ovaries present	NCCN Occult Primary Version 3.2023 (NCCN, 2023w)
CA-125		Lynch Syndrome: surveillance/prevention strategies. CA-125 is an additional ovarian screening test with caveats similar to transvaginal ultrasound	Genetic/Familial High-Risk Assessment: Colorectal Version 2.2022 (NCCN, 2022b)
CA-125		CA-125 may be considered in the initial evaluation of malignant peritoneal mesothelioma	NCCN Mesothelioma: Peritoneal Version 1.2023 (NCCN, 2023n)
CEA		Colon cancer appropriate for resection (non-metastatic) workup and surveillance every 3-6 months for 2 years, then every 6 months for a total of 5 years for stages II-III cancer. Not recommended beyond 5 years	NCCN Colon Cancer Version 3.2023 (NCCN, 2023f)

		Suspected or proven metastatic synchronous adenocarcinoma workup Pedunculated or sessile polyp with invasive cancer but fragmented specimen/unknown margin or unfavorable histology	
CEA		Workup and surveillance for small bowel adenocarcinoma	NCCN Small Bowel Adenocarcinoma Version 1.20232 (NCCN, 2023ac)
CEA		Pedunculated or sessile polyp with invasive cancer but fragmented specimen/unknown margin or unfavorable histology Workup, monitoring, and/or surveillance for rectal cancer	Rectal Cancer Version 4.2022 (NCCN, 2023ab)
CEA		Intrahepatic (such as isolated intrahepatic mass) and extrahepatic cholangiocarcinoma workup and monitoring. CEA is a baseline test and should not be done to confirm diagnosis	NCCN Hepatocellular Carcinoma Version 1.2022 (NCCN, 2023k)
CEA		Gallbladder cancer initial workup (on findings such as jaundice or a mass found on imaging) and surveillance. CEA is a baseline test and should not be done to confirm diagnosis	NCCN Hepatocellular Carcinoma Version 1.2022 (NCCN, 2023k)
CEA		Thyroid carcinoma – medullary carcinoma diagnostic procedure, additional workup, and surveillance; surveillance 2-3 months postoperatively, then every 6-12 mo.	NCCN Thyroid Carcinoma Version 3.2022 (NCCN, 2023aj)
CEA		May be clinically indicated in the workup and management of epithelial ovarian, fallopian tube, primary peritoneal, and less common ovarian cancers	NCCN Ovarian Cancer Version 1.2023 (NCCN, 2023x).

Serum Tumor Markers for Malignancies, continued

CEA		Initial diagnostic evaluation for Malignant pleural or peritoneal mesothelioma	NCCN Mesothelioma: Peritoneal Version 1.2023 (NCCN, 2023n) and Mesothelioma: Pleural Version 1.2023 (NCCN, 2023o)
CEA		Monitoring of metastatic breast cancer and definition of disease progression. An isolated increase in tumor marker should rarely be used as the only indicator of disease progression.	NCCN Breast Cancer Version 3.2023 (NCCN, 2023c)
CEA		Neuroendocrine and Adrenal Tumors - Multiple Endocrine Neoplasia, Type 2: initial diagnostic evaluation and/or surveillance	NCCN Neuroendocrine and Adrenal Tumors Version 2.2022 (NCCN, 2023u)
CEA		Initial workup for non-small cell lung cancer	NCCN Non-Small Cell Lung Cancer Version 2.2023 (NCCN, 2023v)
CEA		Commonly used immunohistochemistry marker for unknown primary cancers. Positive marker for hepatocellular carcinoma. Useful for mesothelioma, thyroid carcinoma (medullary carcinoma).	NCCN Occult Primary Version 3.2023 NCCN (NCCN, 2023w)
Calcitonin (CALCA)		Thyroid carcinoma - medullary carcinoma basal evaluation, post-surgical evaluation, and surveillance (surveillance every 6-12 mo.)	NCCN Thyroid Carcinoma Version 3.2022 (NCCN, 2023aj)
Calcitonin (CALCA)		Cervical cancer - workup	NCCN Cervical Cancer Version 1.2023 (NCCN, 2023d)
Calcitonin (CALCA)		Multiple endocrine neoplasia, type 2 – clinical evaluation	NCCN Neuroendocrine and Adrenal Tumors Version 2.2022 (NCCN, 2023u)
Calcitonin (CALCA)		Workup for occult primary adenocarcinoma or anaplastic/undifferentiated	NCCN Head and Neck Cancers Version 1.2023 (NCCN, 2023j)

		tumors of the head and neck, or otherwise unspecified	
Serum Free-light chain (FLC) assay		Plasma cell disorders, such as myelomas, immunoglobulin light chain amyloidosis, B-cell lymphoma, Waldenström's macroglobulinemia/ lymphoplasmacytic lymphoma, and solitary plasmocytoma—included in initial diagnostic workup; surveillance (as needed) for multiple myeloma	NCCN Ovarian Cancer Version 1.2022 (NCCN, 2022r).NCCN Multiple Myeloma Version 3.2023 (NCCN, 2023p), B-Cell Lymphomas Version 2.2023 (NCCN, 2023a), Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae), Waldenström's macroglobulinemia/ lymphoplasmacytic lymphoma Version 1.2023 (NCCN, 2023al)
Inhibin (INHA)		May be clinically indicated in the workup and management of an undiagnosed pelvic mass, epithelial ovarian, fallopian tube, primary peritoneal, and less common ovarian cancers	NCCN Occult Primary Version 3.2023 (NCCN, 2023w)
Inhibin (INHA)		Commonly used immunohistochemistry marker for unknown primary cancers. Positive marker for adrenocortical carcinoma.	NCCN Occult Primary Version 3.2023 (NCCN, 2023w)
Inhibin (INHA)		Uterine Sarcoma – additional confirmatory tests	NCCN Uterine Neoplasms Version 1.2023 (NCCN, 2023ak)
LDH		May be clinically indicated in the workup and management of epithelial ovarian, fallopian tube, primary peritoneal, and less common ovarian cancers	NCCN Ovarian Cancer Version 1.2023 (NCCN, 2023x)
LDH		Acute Lymphoblastic Leukemia (ALL) workup as part of a tumor lysis syndrome panel; useful for	NCCN Acute Lymphocytic Leukemia Version

		workup under certain circumstances; essential for Richter's transformation	1.2022 (NCCN, 2022a)
LDH		Pediatric Acute Lymphoblastic Leukemia (ALL) workup as part of a tumor lysis syndrome panel	NCCN Pediatric Acute Lymphoblastic Leukemia Version 2.2023 (NCCN, 2023z)
LDH		Chronic lymphocytic leukemia workup for histologic transformation (Richter's) and progression; workup for tumor lysis syndrome. Listed as "essential"	NCCN Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Version 2.2023 (NCCN, 2023e)
LDH		Chronic lymphocytic leukemia workup for histologic transformation (Richter's) and progression; workup for tumor lysis syndrome. Listed as "useful under certain circumstances"	NCCN Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Version 2.2023 (NCCN, 2023e)
LDH		Hairy cell leukemia workup	NCCN Hairy Cell Leukemia. Version 1.2023 (NCCN, 2023i)
LDH		Prognostic factor, 1.5 times the upper limit of normal amount; included in the initial workup for kidney cancer	NCCN Kidney Cancer. Version 4.2023
LDH		Prognostic factor, metastatic disease. Utilized also for stage IV metastatic workup to predict outcomes.	NCCN Melanoma: Cutaneous. Version 2.2023 (NCCN, 2023g)
LDH		Workup and/or surveillance may be indicated for: Follicular lymphoma, Extranodal marginal zone B-cell lymphoma, Gastric and non-gastric MALT, Nodal marginal zone lymphoma, Splenic marginal zone lymphoma, Mantle cell lymphoma, Diffuse large B-cell lymphoma, Burkitt	NCCN B-cell Lymphomas Version 2.2022 (NCCN, 2023a), Hodgkin Lymphoma Version 2.2023 (NCCN, 2023l)

		lymphoma, AIDS-related B-cell lymphomas, Post-transplant lymphoproliferative disorders, Castleman's disease, lymphoblastic lymphoma. Workup for Hodgkin Lymphoma	
LDH		Testicular cancer workup (for "suspicious testicular mass").; LDH, along with other tumor markers are critical in diagnosing GCTs, determining prognosis and assessing treatment outcomes	NCCN Testicular Cancer Version 1.2023 (NCCN, 2023ah)
LDH		Osteosarcoma and Ewing Sarcoma workup and/or surveillance. Elevated levels at initial diagnosis and initial recurrence are considered adverse prognostic indicators. ALP measurement may have clinical relevance and should be done before treatment.	NCCN Bone Cancer Version 2.2023 (NCCN, 2023b)
LDH		Multiple Myeloma initial workup, follow-up/surveillance and staging	NCCN Multiple Myeloma Version 3.2023 (NCCN, 2023p)
LDH		Initial Workup for Systemic Light Chain Amyloidosis	NCCN Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae)
LDH		Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma essential workup	NCCN Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma Version 1.2023 (NCCN, 2023al)
LDH		Initial evaluation for myelodysplastic syndromes or in the workup of suspected myeloproliferative neoplasms; initial evaluation for cytopenia and myelodysplasia	NCCN Myelodysplastic Syndromes Version 3.2023 (NCCN, 2023q) Myeloproliferative Neoplasms Version

Serum Tumor Markers for Malignancies, continued

		3.2022 (NCCN, 2023s)
LDH	Prognostic factor, "one of the most important"	NCCN Small Cell Lung Cancer Version 3.2023 (NCCN, 2023ad)
LDH	Essential workup for primary cutaneous lymphomas	NCCN Primary Cutaneous Lymphoma Version 1.2023 (NCCN, 2023aa)
LDH	T-cell lymphomas – workup, prognosis (except Breast Implant-Associated ALCL). May be used in staging breast implant-associated ALCL	NCCN T-Cell Lymphomas Version 2.2022 (NCCN, 2022x)NCCN T-Cell Lymphomas Version 1.2023 (NCCN, 2023ag)
Troponin T	Diagnostic Workup and staging for systemic light chain amyloidosis	Systemic Light Chain Amyloidosis Version 2.2023 (NCCN, 2023ae)
Tryptase	Serum total tryptase is a WHO diagnostic criterion for systemic mastocytosis. Serum tryptase may also be useful in risk classification and monitoring response to therapy	NCCN Systemic Mastocytosis Version 2.2022 (NCCN, 2023af)
Tryptase	Myeloid/Lymphoid Neoplasms with Eosinophilia and Tyrosine Kinase Fusion Genes - Initial evaluation	NCCN Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase fusion genes Version 1.2022 (NCCN, 2022n)NCCN Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase fusion genes Version 2.2022 (NCCN, 2023r)

The NCCN guidelines on ovarian cancer (version 2.2020) also stated that certain biomarker panels such as OVA1, ROMA (Risk of Ovarian Malignancy Algorithm) and OVERA have been approved by the FDA for "estimating risk of ovarian cancer in women with an adnexal mass for which surgery is planned, and have not yet been referred to an oncologist". However, "currently, the NCCN Panel does not recommend the use of these biomarkers for determining the status of an undiagnosed adnexal/pelvic mass." The NCCN further notes that CA-125, HE4, mesothelin, B7-H4, decoy receptor 3 (DcR3), and spondin-2 "do not increase early enough to be useful in detecting early-stage ovarian cancer" (NCCN, 2023x) .

Monitoring of disease relapse with any tumor markers is not recommended for breast cancer. Ki-67 is not recommended for any assessment of breast cancer. Testing for immunohistochemical markers such as ER/PR and HER2 is recommended for assessment of breast cancer (NCCN, 2023c).

Cardiac biomarkers such as troponin I or T and BNP (or NT-proBNP) may be considered important predictors of outcome of systemic light chain amyloidosis (NCCN, 2023ae).

The 2020 NCCN Guidelines on Hepatocellular Carcinoma states, "serum biomarkers such as AFP may incrementally improve the performance of imaging-based screening and surveillance, but their cost effectiveness has not been established; their use as supplementary surveillance tests is optional." (NCCN, 2023k)

For Merkel Cell Carcinoma, the NCCN stated a neuroendocrine marker such as chromogranin, CD56, neurofilament protein, neuron-specific enolase, or synaptophysin may be used (NCCN, 2023m). Small cell lung cancers (SCLCs) also stain positive for biomarkers of neuroendocrine differentiation but cannot be used to differentiate SCLCs from NSCLCs (NCCN, 2023m).

NCCN recommends against using OVA1 or OvaSure as screening for ovarian cancer (NCCN, 2023t, 2023x).

International Mesothelioma Interest Group

The Interest Group considers the following biomarkers to be "very useful": Calretinin Cytokeratin 5/6, WT1, Podoplanin (D2-40) (for epitheloid mesothelioma), Claudin 4, MOC31, B72.3, CEA, BER-EP4, BG8 (Lewis^{Y}), TTF-1, and Napsin A (for lung adenocarcinoma) (Husain et al., 2018).

North American Neuroendocrine Tumor Society (NANETS)

NANETS notes that although most of its expert panel's members measure CgA and/or pancreastatin, a majority of them believed that "these tumor markers assist in patient management only occasionally or rarely". No consensus was reached on whether these tumor markers should be routinely measured (NANETS, 2017).

In 2020, NANETS published a guideline focusing on the "Surveillance and Medical Management of Pancreatic Neuroendocrine Tumors". In it, they remark that "Use of nonspecific tumor markers such as CgA, pancreastatin (PcSt), and others is not recommended for routine use in patients with pNETs", stating that these marker analyses "rarely, if ever" influence treatment (Halfdanarson et al., 2020)

American Thyroid Association (ATA)

The ATA cannot recommend for or against routine measurement of serum calcitonin in patients with thyroid nodules. Furthermore, the ATA cautions that unusual levels of calcitonin may occur with a variety of other conditions apart from MTC, and notes that calcitonin levels are often elevated in young children and males compared to females (Haugen et al., 2016; Wells et al., 2015).

American Association for the Study of Liver Diseases

The AASLD states that ultrasound surveillance for hepatocellular carcinoma may be done with or without AFP, every 6 months. However, other biomarkers apart from AFP need more research and validation (AASLD, 2018).

European Association for the Study of the Liver (EASL)

EASL stated "Tumour biomarkers for accurate early detection are still lacking. The data available show that the biomarkers tested (i.e. AFP, AFP-L3 and DCP) are suboptimal in terms of cost-effectiveness for routine surveillance of early HCC (evidence low) (EASL, 2018)."

American College of Radiology (ACR)

The ACR recommends against screening serum markers for the diagnosis of hepatic fibrosis and cirrhosis disease and disease progression, stating that "although a variety of serum markers exist for this purpose, they are inaccurate for intermediate stages of fibrosis, and imaging by conventional ultrasound (US), CT, and MRI is frequently performed to assess for cirrhosis and its complications in this patient population"(ACR, 2019).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

There are numerous FDA-approved tests for the assessment of serum tumor markers. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81500	Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE4), utilizing serum, with menopausal status, algorithm reported as a risk score Proprietary test: Risk of Ovarian Malignancy Algorithm (ROMA)™ Lab/manufacturer: Fujirebio Diagnostics
81503	Oncology (ovarian), biochemical assays of five proteins (CA-125, apolipoprotein A1, beta-2 microglobulin, transferrin, and pre-albumin), utilizing serum, algorithm reported as a risk score Proprietary test: OVA1™ Lab/manufacturer: Vermillion, Inc
81538	Oncology (lung), mass spectrometric 8-protein signature, including amyloid A, utilizing serum, prognostic and predictive algorithm reported as good versus poor overall survival Proprietary test: VeriStrat® Lab/manufacturer: Biodesix, Inc
82105	Alpha-fetoprotein (AFP); serum
82107	Alpha-fetoprotein (AFP); AFP-L3 fraction isoform and total AFP (including ratio)
82232	Beta-2 microglobulin
82308	Calcitonin
82378	Carcinoembryonic antigen (CEA)

83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83521	Immunoglobulin light chains (ie, kappa, lambda), free, each
83615	Lactate dehydrogenase (LD), (LDH)
83880	Natriuretic peptide
83950	Oncoprotein; HER-2/neu
83951	Oncoprotein; des-gamma-carboxy-prothrombin (DCP)
84075	Phosphatase, alkaline
84078	Phosphatase, alkaline; heat stable (total not included)
84080	Phosphatase, alkaline; isoenzymes
84484	Troponin, quantitative
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
85415	Fibrinolytic factors and inhibitors; plasminogen activator
86300	Immunoassay for tumor antigen, quantitative; CA 15-3 (27.29)
86301	Immunoassay for tumor antigen, quantitative; CA 19-9
86304	Immunoassay for tumor antigen, quantitative; CA 125
86305	Human epididymis protein 4 (HE4)
86316	Immunoassay for tumor antigen, other antigen, quantitative (e.g., CA 50, 72-4, 549), each
86336	Inhibin A
0003U	Oncology (ovarian) biochemical assays of five proteins (apolipoprotein A-1, CA 125 II, follicle stimulating hormone, human epididymis protein 4, transferrin), utilizing serum, algorithm reported as a likelihood score Proprietary test: Overa™ (OVA1 Next Generation) Lab/manufacturer: Aspira Labs, Inc, Vermillion, Inc
0092U	Oncology (lung), three protein biomarkers, immunoassay using magnetic nanosensor technology, CPTsma, algorithm reported as risk score for likelihood of malignancy Proprietary test: REVEAL Lung Nodule Characterization Lab/Manufacturer: MagArray, Inc
0163U	Oncology (colorectal) screening, biochemical enzyme-linked immunosorbent assay (ELISA) of 3 plasma or serum proteins (teratocarcinoma derived growth factor-1 [TDGF-1, Cripto-1], carcinoembryonic antigen [CEA], extracellular matrix protein [ECM]), with demographic data (age, gender, CRC-screening compliance) using a proprietary algorithm and reported as likelihood of CRC or advanced adenomas Proprietary test: BeScreened™-CRC Lab/Manufacturer: Beacon Biomedical Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes
8/22/22	Updated criteria with the following changes: Addition of note above criteria on frequency: "Note: Except for where otherwise specified in the coverage criteria below, quarterly measurement of designated serum tumor markers is permitted for follow-up, monitoring, and/or surveillance" CC1 totally reformatted and reworded to meet current guidelines. Addition of two new biomarkers in CC1: c) B-type natriuretic peptide (BNP) or N-terminal fragment of B-type natriuretic peptide (NTproBNP) i) Systemic light chain amyloidosis: initial diagnostic evaluation j) Free light chain (circulating serum kappa or lambda chains) for: i) B-cell lymphoma – Castleman disease: initial diagnostic evaluation ii) Multiple myeloma: initial diagnostic evaluation and/or surveillance as needed iii) Systemic light chain amyloidosis: initial diagnostic evaluation iv) Waldenström's Macroglobulinemia/ Lymphoplasmacytic Lymphoma: initial diagnostic evaluation

	CC4- OvaSure and Coloprint removed from list, as these tests are no longer on the market. Also, added CPT codes 83521 and 83880.
10/27/23	The following changes were implemented: Reorganized coverage criteria #1 such that the focus is the cancer and then all the appropriate biomarkers vs the previous organization where the focus was the biomarker and then all the cancer indications. Removed coverage criteria #2, thereby creating new coverage criteria #2 and #3, which were both modified from previous #3 and #4, now read: "2) For all other cancer indications not discussed above, use of the above biomarkers (alone or in a panel of serum tumor markers) **DOES NOT MEET COVERAGE CRITERIA**. 3) All other serum tumor markers not addressed above (alone or in a panel of serum tumor markers) **DO NOT MEET COVERAGE CRITERIA**."

G2124 Serum Tumor Markers for Malignancies

Testing for Alpha-1 Antitrypsin Deficiency

Policy #: AHS – M2068	**Prior Policy Name & Number (as applicable):** M2068 Genetic Testing for Alpha-1 Antitrypsin Deficiency
Implementation Date: 9/15/21	**Date of Last Revision:** 8/19/22 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Alpha 1-antitrypsin deficiency (AATD) is a genetic disease that causes deficient or defective production of the alpha-1 antitrypsin (AAT) protease inhibitor that can affect the lungs, liver, and skin (Stoller, 2020b). AAT deficiency results in unbalanced rapid breakdown of proteins, especially in the supporting elastic tissue of the lungs (NORD, 2022).

II. Related Policies

Policy Number	Policy Title
	Not applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

Serum Testing

1. Serum quantification of alpha-1 antitrypsin (A1AT) protein and/or A1AT phenotyping by isoelectric focusing or A1AT proteotyping (Pi-typing or protease inhibitor typing) for Z and S alleles by liquid chromatography-tandem mass spectrometry MEETS COVERAGE CRITERIA in the following situations:

a. Symptomatic adults with emphysema, COPD or asthma

b. Individuals with unexplained liver disease

c. Individuals with persistent obstruction on pulmonary function tests without identifiable risk factors (e.g. cigarette smoking, occupational exposure)

d. Adults with necrotizing panniculitis

e. Siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency

f. Individuals with anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis)

g. Individuals with bronchiectasis without evident etiology

2. In individuals with negative genotype testing for common variants or discordant results between A1AT serum levels and proteotype, isoelectric focusing/phenotyping **MEETS COVERAGE CRITERIA** when there is strong suspicion of the disease based on laboratory testing and symptoms.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3. Testing for alpha-1 antitrypsin deficiency **DOES NOT MEET COVERAGE CRITERIA** in all other situations.

Note:

In 2003, the American Thoracic Society published recommendations on the diagnosis and management of individuals with AAT deficiency.

Recommendations were classified as follows:

- Type A: Genetic testing is recommended
- Type B: Genetic testing should be discussed and could be accepted or declined
- Type C: Genetic testing is not recommended, i.e., should not be encouraged
- Type D: Recommend against genetic testing, i.e., should be discouraged

Type A recommendations for diagnostic testing in the following situations:

1. Symptomatic adults with emphysema, COPD or asthma with airflow obstruction that is not completely reversible with aggressive treatment with bronchodilators

2. Individuals with unexplained liver disease

3. Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure)
4. Adults with necrotizing panniculitis
5. Siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency

Type B recommendations for diagnostic testing in the following situations:

1. Adults with bronchiectasis without evidence etiology
2. Adolescents with persistent airflow obstruction
3. Asymptomatic individuals with persistent airflow obstruction and no risk factors
4. Adults with C-ANCA positive (anti-proteinase 3-positive) vasculitis
5. Individuals with a family history of COPD or liver disease not known to be attributed to AAT deficiency
6. Distant relatives of an individual who is homozygous for AAT deficiency
7. Offspring or parents of an individual with homozygous AAT deficiency
8. Siblings, offspring, parents or distant relatives of an individual who is heterozygous for AAT deficiency
9. Individuals at high risk of having AAT deficiency-related diseases
10. Individuals who are not at risk themselves of having AAT deficiency but who are partners of individuals who are homozygous or heterozygous for AAT deficiency

Type C recommendations for diagnostic testing in the following situations

1. Adults with asthma in whom airflow obstruction is completely reversible
2. Predispositional testing
3. Population screening of smokers with normal spirometry

Type D recommendations for diagnostic testing in the following situations:

1. Predispositional fetal testing
2. Population screening of either neonates, adolescents, or adults*

* Population screening is not recommended currently. However, a possible exception (type B recommendation) may apply in countries satisfying all three of the following conditions: (1) the prevalence of AAT deficiency is high (about 1/1,500, or more); (2) smoking is prevalent; and (3) adequate counseling services are available.

According to the 2003 joint statement on diagnosis and management of alpha-1 antitrypsin deficiency by the American Thoracic Society/European Respiratory Society: (1)

The following features should prompt suspicion by physicians that their patient may be more likely to have AAT deficiency:

Clinical Factors

1. Early-onset emphysema (age of 45 years or less)
2. Emphysema in the absence of a recognized risk factor (smoking, occupational dust exposure, etc.)
3. Emphysema with prominent basilar hyperlucency
4. Otherwise unexplained liver disease
5. Necrotizing panniculitis
6. Anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis)
7. Bronchiectasis without evident etiology

IV. Scientific Background

Alpha-1 antitrypsin (AAT) deficiency is an underrecognized genetic condition that affects approximately 1 in 2,000 to 1 in 5,000 individuals and predisposes to liver disease and early-onset emphysema (Stoller & Aboussouan, 2012). It is estimated (Campos, Wanner, Zhang, & Sandhaus, 2005) that up to 80,000 to 100,000 people in the United States have the severe form of the disease (homozygous in null or abnormal alleles). There is much variation in the disease prevalence in other nations (de Serres, Blanco, & Fernandez-Bustillo, 2007), but most current estimates are that 3 million people worldwide have severe AATD (Stoller, 2020a).

Alpha-1 Antitrypsin deficiency (AATD) is a result of abnormal alpha-1 antitrypsin (AAT) protein inherited in an autosomal recessive pattern with codominant expression in which both genes inherited can be active and contribute to the genetic trait they control. AAT is a member of the serine protease inhibitor (Pi) family, referred to as "serpins", and it inhibits the proteolytic enzymes elastase, trypsin, chymotrypsin, and thrombin. AAT is encoded by the gene *SERPINA1* (Stoller, 2020a).

The AAT protein is produced in the liver and has a role in protecting lungs from injury by neutrophil elastase, which is secreted by white blood cells as a response to inflammation or infection. If the enzyme remains unchecked by AAT protein, damage to alveoli resulting in chronic obstructive pulmonary disease can occur. This includes emphysema, asthma, bronchiectasis, and spontaneous pneumothorax. Smoking and other environmental exposure can cause further damage (J. Stoller, 2020a, 2020b).

Abnormal molecules of AAT protein caused by this illness can also cause liver dysfunction. Pathologic polymerization of the variant AAT can occur, resulting in intrahepatocyte accumulation of AAT molecules, leading to cirrhosis, fibrosis, cholestasis, or hepatomegaly. Liver disease is more common in individuals with certain allele combinations. Male gender and obesity may be risk factors for progression to advanced liver disease in adulthood among patients with severe AAT deficiency. In contrast, alcohol use and viral hepatitis do not appear to increase the risk of progressive hepatic failure (J. Stoller, 2020b). AATD is a common genetic cause of liver disease in children (de Serres, Blanco, & Fernandez-Bustillo, 2003).

Skin manifestations of AATD are also recognized. The most associated skin condition is necrotizing panniculitis. In this condition, inflammatory skin lesions are thought to be a consequence of the AAT protein loss of function and subsequent unchecked proteolysis enzyme activity in the skin and subcutaneous tissue. Associations between alpha-1 antitrypsin (AAT) and vascular disease, inflammatory bowel disease, glomerulonephritis, and vasculitis have been proposed but not definitively established (J. Stoller, 2020b).

Due to the numerous alleles associated with AAT, each allele has been given a letter code based on the "electrophoretic mobility of the protein produced". The normal allele is the "M" allele, and the most common mutation is the "Z" allele. This system applies for each individual allele; for example, a homozygous Z genotype would be denoted as "ZZ". Similarly, a wildtype (or "normal") genotype would be "MM". Besides the normal phenotype, the three other categories of AAT include "deficient" in which insufficient AAT is produced; "null" in which no AAT is produced at all; and "dysfunctional" in which a typical amount of AAT is produced, but the AAT protein does not function correctly (J. Stoller, 2020a).

Proprietary Testing

Initial testing often begins with serum quantification of AAT protein. This can be done through several methods, including immune turbidimetry and nephelometry (Stoller, 2020a). A low level is generally represented by a serum level below 11 micromol/L (less than 57 mg/dl using nephelometry). Due to the variation of reference ranges in different testing methodologies, most labs will complete isoelectric phenotyping on any individual with a serum AAT levels of < 100 mg/dL (18.4 micromol/L). In fact, the American Thoracic Society suggests persons with borderline serum levels (defined as 12-35 micromoles or 90 to 140 mg/dL) have qualitative testing (ATS/ERS, 2003).

Isoelectric immunophenotype testing uses the difference in migration rates of allele variants under isoelectric focusing. For example, the M variant will migrate to the middle of the gel, Z will migrate the slowest, and F migrates quickly to the side closest to the anode. This is not a genetic test. On occasion the results can be inconclusive or discordant with quantitative testing, requiring genotype testing of the most-common variants (J. Stoller, 2020a).

Genotype testing for the most common allele variants can be utilized where isoelectric immunophenotype testing is inconclusive. Usually polymerase chain reaction (PCR) or restriction fragment length polymorphism (RFLP) techniques are utilized to determine if the most common alleles are present. When dealing with the possibility of a rare variant or null allele, full gene sequencing can be utilized as a final diagnostic measure (J. Stoller, 2020a).

In 2017, Grifols won FDA approval for AAT Deficiency Test, which is capable of simultaneously analyzing 99% of the most prevalent known mutations causing alpha-1 antitrypsin deficiency. The molecular test analyzes simultaneously 192 samples per kit, and in a single reaction, identifies 14 of the most prevalent known mutations in the SERPINA1 gene, responsible for this genetic disorder.

Using Progenika's FDA-cleared A1AT Genotyping Test, Matrix Clinical Labs released the proprietary Alpha ID screening test, a comprehensive targeted genetic test assessing 14 common and rare alleles in the *SERPINA1* gene. "The Alpha ID screening test utilizes an easy-to-use saliva collection swab and does not require a blood draw or finger stick. If a positive result is found using this test, a follow-up test, Alpha ID Confirm, uses a finger stick and a blood spot card to asses A1AT protein levels as well a potential reflex to next-generation sequencing (NGS) to help physicians achieve an accurate diagnosis of Alpha-1 antitrypsin deficiency (A1ATD)" (Matrix Clinical Labs & 2022).

Clinical Utility and Validity

The literature on the analytic and clinical validity of genetic testing for AATD is limited. In addition, few randomized controlled trials (RCTs) have evaluated the impact of AATD testing on patient outcomes. Current evidence-based guidelines (Vogelmeier et al., 2017) for diagnosis and management of AATD recommend specific interventions for patients with emphysema and AATD. AAT augmentation therapy is often prescribed for patients with AATD and chronic obstructive pulmonary disorder (COPD). In addition, several studies have documented that the disease is under-recognized with delay in diagnosis of between 5 to 8 years (Barrecheguren et al., 2016; Stoller et al., 2005).

Snyder et al. (2006) evaluated the laboratory methods of assessing AATD. Samples from 512 individuals were analyzed, and "A1AT concentrations were measured by nephelometry. Phenotype analysis was performed by isoelectric focusing electrophoresis. The genotype assay detected the S and Z deficiency alleles by a melting curve analysis." Of these 512 samples, 10 (2%) were discordant between genotype and phenotype. Of these 10 results, 7 were attributed to phenotyping errors. 4% of the samples submitted to genotype and quantitative analysis were "reflexed" to phenotyping, where phenotyping confirmed the genotype result 85% of the time. The investigators concluded, "The combination of genotyping and quantification, with a reflex to phenotyping, is the optimal strategy for the laboratory evaluation of A1AT deficiency (Snyder et al., 2006)."

Sorroche et al. (2015) examined a cohort of COPD patients and the prevalence of severe AATD. 1002 patients were evaluated, and 785 (78.34%) had normal AAT levels. The remaining 217 patients had low AAT levels, but only 15 patients had a genotype associated with severe AATD. Of these 15 patients, 12 were ZZ and 3 were SZ. Of the 202 other patients, 29 were a Z heterozygote, 25 were an S heterozygote, and 4 were an SS homozygote. 144 patients could not be definitively diagnosed (Sorroche et al., 2015).

Corda et al. (2011) examined the prevalence of AATD in a supposed "high-risk" area. 817 residents participated, and 67 had low AAT serum levels. 118 residents carried AATD-related alleles, 114 of which were heterozygotes "(46 Z, 52 S, 9 P(brescia), 4 M(wurzburg), 2 I, 1 P(lowell)". The authors concluded, "the large number of mostly asymptomatic individuals with AATD identified suggests that in high-risk areas adult population screening programs employing the latest genetic methods are feasible (Corda et al., 2011)."

Soriano et al. (2018) evaluated the prevalence of AATD testing in COPD patients. The patient sample came from "550 UK Optimum Patient Care Research Database general practices". Out of 107,024 COPD patients, only 2.2% had any record of being tested for AATD. Of those tested, 23.7% were diagnosed with AATD. The investigators also noted that between 1994 and 2013, the incidence of AATD diagnosis increased. The authors concluded "that AATD remains markedly underdiagnosed in COPD patients (Soriano et al., 2018)."

Greulich et al. (2016) evaluated the results of a large targeted screening program for AATD. The samples were distributed by a German AAT laboratory over a period of 12 years, and 18,638 testing kits were obtained. Of this sample, 6919 carried at least one mutation, and 1835 patients were considered to have severe AATD. 194 of these patients had "rare" genotypes. The authors concluded that "among clinical characteristics, a history of COPD, emphysema, and bronchiectasis were significant predictors for Pi*ZZ, whereas a history of asthma, cough and phlegm were predictors of not carrying the genotype Pi*ZZ (Greulich et al., 2016)."

Mattman et al. (2020) compared the comprehensiveness and efficiency of pathogenic variant (PV) detection of four different protocols from 2011 to 2018 in laboratories across Canada. From 5399 index patients, 396 ZZ genotypes were identified. The protocol for serum A1AT concentration/DNA sequencing in the Ontario center (ON-CD) yielded the highest PV detection – "genotypes with at least one PV, other than S, Z, or F, were identified at 0.67/ZZ as compared to <0.2/ZZ (all others)." However, it also had the highest rates of undefined molecular variants (UMV) (0.16/ZZ vs <0.12/ZZ) or likely benign variants (LBV) compared to all others (0.08/ZZ vs <0.06/ZZ). The authors concluded the "strategies with readily detect variants across the full coding sequence of *SERPINA1* detect more PV as well as more UMV and LBV" (Mattman et al., 2020).

Hamesch et al. (2019) evaluated the clinical landscape of liver symptoms in patients with AATD, specifically the Pi*ZZ genotype. 554 patients (403 exploratory cohort, 151 confirmatory cohort) were included and were compared to 234 controls without pre-existing liver disease. The authors found significantly higher levels of serum liver enzymes in the Pi*ZZ carriers compared to controls, further noting that "significant' fibrosis was suspected in 20%-36% of Pi*ZZ carriers. Signs of advanced fibrosis were 9 to 20 times more common in carriers compared to non-carriers. Controlled attenuation parameter of ≥280 dB/m, which suggests "severe" steatosis was detected in 39% of carriers compared to 31% of controls. Finally, Pi*ZZ carriers were found to have lower serum concentrations of triglyceride, low, and very-low density lipoprotein cholesterol compared to controls, which the authors suggested to represent impaired hepatic secretion of liquid. Overall, the authors concluded that they identified evidence of liver steatosis, impaired liver secretion, liver fibrosis, and that their data could assist in hepatologic management of Pi*ZZ carriers (Hamesch et al., 2019).

Strnad et al. (2019) investigated the impact of the Pi*Z and Pi*S genotypes on subjects with non-alcoholic fatty liver disease (NAFLD) or alcohol misuse. Separate cohorts of 1184 with NAFLD and 2462 with chronic alcohol abuse were included. The authors found Pi*Z genotypes in 13.8% of patients with cirrhotic NAFLD but only 2.4% of patients without liver fibrosis. From there, the increased risk of NAFLD subjects to develop cirrhosis was found to be 7.3 times higher in Pi*Z carriers. The Pi*Z variant was also found in 6.2% of alcohol abusers but only 2.2% of alcohol abusers without significant liver injury. The

increased risk was found to be 5.2 times higher in Pi*Z carriers. The Pi*S variant was not associated with NAFLD-related cirrhosis and only mildly with alcohol-related cirrhosis (increased risk = 1.47 times). The authors concluded that the Pi*Z variant was the strongest "single nucleotide polymorphism-based risk factor for cirrhosis in NAFLD and alcohol misuse, whereas the Pi*S variant confers only a weak risk in alcohol misusers" and remarked that this finding should be considered in future genetic counseling of affected individuals (Strnad et al., 2019).

Carreto et al. (2020) examined the utility of routine screening for AATD among patients with bronchiectasis, due to the contradiction in guidelines from the British Thoracic Society, which recommend screening for bronchiectasis among patients with AATD, but not vice versa. After screening 1600 patients with bronchiectasis from two centers in the UK from 2012-2016, they found only eight patients with AATD. They concluded that because of the low prevalence of AATD as an etiology for disease presentation among patients with bronchiectasis, routine screening for AATD would not significantly impact clinical management through augmentation therapy, smoking cessation, and genetic counselling, among other methods. Despite this, the researchers did note that higher rates of detection may be found in other geographical regions in the UK or in other countries (Carreto et al., 2020).

(Bellemare et al., 2021) studied the clinical utility of determining the allelic background of mutations causing alpha-1 antitrypsin deficiency. *SERPINA1* was DNA sequenced to identify rare variants that could confer the risk of developing emphysema. Seven carriers of a rare variant, Leu353Phe_fsTer24, known to lead to undetectable serum levels of AAT, were studied using an allele-specific DNA sequencing method that they developed. Results demonstrated that Leu353Phe_fsTer24 variant was transmitted on the same allele as the M3 variant in all the patients and two of the seven patients had either a S or Z allele. The lowest AAT serum levels were observed in compound heterozygotes for the S or Z allele, suggesting higher risk of developing emphysema. This study showed that understanding the clinical significance of genetic variants found in SERPINA1 can lead to better clinical outcomes (Bellemare et al., 2021).

V. Guidelines and Recommendations

American Thoracic Society/European Respiratory Society (ATS/ERS)

The ATS/ERS released joint guidelines on the "Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency." These recommendations are as follows (ATS/ERS, 2003):

Policy Guidelines

Recommendations were classified as follows:

- Type A: Genetic testing is recommended
- Type B: Genetic testing should be discussed and could be accepted or declined
- Type C: Genetic testing is not recommended, i.e., should not be encouraged
- Type D: Recommend against genetic testing, i.e., should be discouraged

Testing for Alpha-1 Antitrypsin Deficiency, continued

Type A recommendations for diagnostic testing in the following situations:

1. Symptomatic adults with emphysema, COPD or asthma with airflow obstruction that is not completely reversible with aggressive treatment with bronchodilators
2. Individuals with unexplained liver disease
3. Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure)
4. Adults with necrotizing panniculitis
5. Siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency

Type B recommendations for diagnostic testing in the following situations:

1. Adults with bronchiectasis without evidence etiology
2. Adolescents with persistent airflow obstruction
3. Asymptomatic individuals with persistent airflow obstruction and no risk factors
4. Adults with C-ANCA positive (anti-proteinase 3-positive) vasculitis
5. Individuals with a family history of COPD or liver disease not known to be attributed to AAT deficiency
6. Distant relatives of an individual who is homozygous for AAT deficiency
7. Offspring or parents of an individual with homozygous AAT deficiency
8. Siblings, offspring, parents or distant relatives of an individual who is heterozygous for AAT deficiency
9. Individuals at high risk of having AAT deficiency-related diseases
10. Individuals who are not at risk themselves of having AAT deficiency but who are partners of individuals who are homozygous or heterozygous for AAT deficiency

Type C recommendations for diagnostic testing in the following situations:

1. Adults with asthma in whom airflow obstruction is completely reversible
2. Predispositional testing
3. Population screening of smokers with normal spirometry

Type D recommendations for diagnostic testing in the following situations:

1. Predispositional fetal testing

2. Population screening of either neonates, adolescents or adults*

* Population screening is not recommended currently. However, a possible exception (type B recommendation) may apply in countries satisfying all three of the following conditions: (1) the prevalence of AAT deficiency is high (about 1/1,500, or more); (2) smoking is prevalent; and (3) adequate counseling services are available.

The following features should prompt suspicion by physicians that their patient may be more likely to have AAT deficiency:

Clinical Factors

1. Early-onset emphysema (age of 45 years or less)
2. Emphysema in the absence of a recognized risk factor (smoking, occupational dust exposure, etc.)
3. Emphysema with prominent basilar hyperlucency
4. Otherwise unexplained liver disease
5. Necrotizing panniculitis
6. Anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis)
7. Bronchiectasis without evident etiology

The ATS/ERS also made statements on serum testing for AATD. "Serum phenotyping by isoelectric focusing performed by a reliable laboratory is the accepted "gold standard" for diagnosing AAT deficiency". The guidelines recommend "that all subjects with COPD or asthma characterized by incompletely reversible airflow obstruction should be tested once for quantitative AAT determination. Also, individuals with evidence of cirrhosis of the liver with no known etiology should be tested for candidate phenotypes (e.g., PI*ZZ, PI*MZ, PI*Mmalton) and testing should be considered in individuals with the syndrome of Wegener's granulomatosis (antiproteinase-3 vasculitis) (ATS/ERS, 2003)."

American College of Gastroenterology (ACG)

The ACG recommends the following for AATD:

- "Patients with persistently elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) should undergo screening for alpha-1 antitrypsin (A1AT) deficiency with alpha-1 anti-trypsin phenotype."
- Evaluation of hepatocellular injury (defined by the guidelines as "disproportionate elevation of AST and ALT levels compared with alkaline phosphatase levels") includes testing for A1AT deficiency (Kwo et al., 2017)".

World Health Organization (WHO)

The WHO released a memorandum on AATD regarding AATD's association with conditions such as COPD and asthma. Their recommendation is as follows: "It is therefore recommended that all patients with COPD and adults and adolescents with asthma be screened once for AAT deficiency using a quantitative test. Those with abnormal results on screening should undergo PI typing" (WHO, 1997).

European Respiratory Society (ERS)

The ERS (Miravitlles et al., 2017) published updated guidelines which recommend:

- "The quantitative determination of AAT levels in blood is a crucial first test to identify AATD. Quantitative deficiency must be supported by qualitative tests to identify the genetic mutation(s) causing AATD."
- "Protein phenotyping by isoelectric focusing identifies variants where AAT is present in the sample including the rarer variants F, I and P *etc*."
- "Genotyping allows a rapid and precise identification/exclusion of S and Z alleles and other variants, where specific primers are available."
- "Gene sequencing remains necessary for those cases where a null variant or a deficient variant other than Z or S is suspected."
- "Testing of relatives of identified patients should be considered after appropriate counselling.
- "Genetic testing should be carried out only after informed consent is given and in accordance with the relevant guidelines and legislation."

The ERS has also noted that "there is no evidence to support efficacy of AAT augmentation therapy in PiSZ, PiMZ or current smokers of any protein phenotype (Miravitlles et al., 2017)."

Alpha-1 Foundation

The Alpha-1 Foundation (Sandhaus et al., 2016) sponsored a medical and scientific advisory committee of experts to examine all relevant, recent literature in order to provide concise recommendations for the diagnosis and management of individuals with AATD.

- "For family testing after a proband is identified, AAT level testing alone is not recommended because it does not fully characterize disease risk from AATD."
- "For diagnostic testing of symptomatic individuals, they recommend genotyping for at least the S and Z alleles. Advanced or confirmatory testing should include Pi-typing, AAT level testing, and/or expanded genotyping."
- "All patients with COPD, unexplained chronic liver disease, necrotizing panniculitis, granulomatosis with polyangiitis, or unexplained bronchiectasis should be tested for AATD."
- "Parents, siblings, and children, as well as extended family of individuals identified with an abnormal gene for AAT, should be provided genetic counseling and offered testing for AATD (see guideline document for special considerations about testing minors)."

The Foundation also noted the following (these statements were not labeled recommendations):

- "For primary diagnosis of AATD the most sensitive and specific method of diagnosis is direct identification of the Z allele by genotyping. By also including the S allele, genotyping for the S and Z allele is greater than 99% specific and sensitive. "
- "AAT levels are insufficient to identify at risk individuals because the AAT level changes with inflammation, pregnancy, and in children. "
- "The range of serum AAT levels among individuals with specific genotypes is sufficiently broad that there is overlap between different genotypes. Thus, serum AAT levels cannot discriminate between different genotypes and additional AAT testing is needed"

Global Initiative for Chronic Obstructive Lung Disease (GOLD)

GOLD notes that genes such as MMP-12 may contribute to a decline in lung function. However, they acknowledge that "it remains uncertain whether these are genes are directly responsible for COPD or are merely markers of causal genes" (GOLD, 2021).

On alpha-1-antitrypsin deficiency, GOLD also stated "Although the classical patient is young (<45 years) with panlobular basal emphysema, it has become recognized that delay in diagnosis has led to identification of some AATD patients when they are older and have a more typical distribution of emphysema (centrilobular apical). A low concentration (<20% normal) is highly suggestive of homozygous deficiency. Family members should be screened and, together with the patient, referred to specialist centers for advice and management" (GOLD, 2021).

Canadian Thoracic Society (CTS)

The CTS released guidelines on genetic testing for AATD, which are as follows:

- "We suggest targeted testing for A1AT deficiency be considered in individuals with COPD diagnosed before 65 years of age or with a smoking history of <20 pack years. (Grade of recommendation: 2C)"
- "We suggest targeted testing for A1AT deficiency not be undertaken in individuals with bronchiectasis or asthma. (Grade of recommendation: 2C) (Marciniuk et al., 2012)"

National Institute Health and Care Excellence (NICE)

NICE published a guideline discussing chronic obstructive pulmonary disease (COPD) in 2019. In it, they note that measurement of serum alpha-1 antitrypsin has a role in identifying deficiencies if the condition is "early onset, [of] minimal smoking history, or [has] family history".

VI. Applicable State and Federal Regulations

Food and Drug Administration

On November 17, 2017, the FDA approved Grifols' (Grifols, 2017) *SERPINA1* Variant Detection System as a qualitative in vitro molecular diagnostic system used to detect variants in *SERPINA1* gene in genomic DNA isolated from human specimens. On November 7, 2019, the FDA approved Grifols' AlphaID™, a cheek swab that can screen patients with COPD for alpha-1 antitrypsin deficiency. It "utilizes an FDA-approved genotyping assay to screen for the 14 most prevalently reported genetic mutations associated

with Alpha-1, including the S, Z, F, I alleles, as well as rare and null alleles, helping detect patients who are at risk for this treatable condition" (Grifols, 2019).

On April 6, 2017 the FDA approved (FDA, 2017) the 23andMe PGS Genetic Health Risk Report for Alpha-1 Antitrypsin Deficiency (AATD) which determines if a person has variants associated with a higher risk of developing AATD-associated lung or liver disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the PI*Z (rs28929474) and PI*S (rs17580) variants in the *SERPINA1* gene by using the 23andMe Personal Genome Service.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81332	*SERPINA1* (serpin peptidase inhibitor, clade A, alpha - 1 antiproteinase, antitrypsin, member 1) (e.g., alpha-1 antitrypsin deficiency), gene analysis, common variants (e.g., *S and *Z)
82103	Alpha-1-antitrypsin; total
82104	Alpha-1-antitrypsin; phenotype
82542	Column chromatography, includes mass spectrometry, if performed (e.g., HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
83789	Mass spectrometry and tandem mass spectrometry (e.g., MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Testing for Diagnosis of Active or Latent Tuberculosis

Policy #: AHS – G2063	**Prior Policy Name & Number (as applicable):** AHS–G2063-201670928 – Gamma Interferon Blood Test for Diagnosis of Latent Tuberculosis
Implementation Date: 9/15/21	**Date of Last Revision:** 11/12/21, 4/3/23 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Infection by *Mycobacterium tuberculosis* (Mtb) results in a wide range of clinical presentations dependent upon the site of infection from classic signs and symptoms of pulmonary disease (cough >2 to 3 weeks' duration, lymphadenopathy, fevers, night sweats, weight loss) to silent infection with a complete absence of signs or symptoms (Lewinsohn et al., 2017).

Culture of Mtb is the gold standard for diagnosis as it is the most sensitive and provides an isolate for drug susceptibility testing and species identification (Bernardo, 2022). Nucleic acid amplification tests (NAAT) use polymerase chain reactions (PCR) to enable sensitive detection and identification of low-density infections (Pai et al., 2004). Interferon-gamma release assays (IGRAs) are blood tests of cell-mediated immune response which measure T cell release of interferon (IFN)-gamma following stimulation by specific antigens such as *Mycobacterium tuberculosis* antigens (Lewinsohn et al., 2017; Dick Menzies, 2022) used to detect a cellular immune response to *M. tuberculosis* which would indicate latent tuberculosis infection (LTBI) (Pai et al., 2014).

II. Related Policies

Policy Number	Policy Title
	Not Applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. To diagnose or screen for latent tuberculosis (TB) infection, an interferon gamma release assay (IGRA) **MEETS COVERAGE CRITERIA.**
   a. Individuals who are at risk for infection with Mycobacteroium tuberculosis (Mtb).
   b. Individuals who are unlikely to be infected with *Mtb* when screening is obliged by law.

2. For all suspected TB infections, the following tests **MEET COVERAGE CRITERA:**

   a) Acid fast bacilli (AFB) smear/stain

b) Culture and culture-based drug susceptibility testing of *Mycobacteria* spp. 3. Direct probe or amplified probe nucleic acid-based testing, including PCR, **MEETS COVERAGE CRITERIA** for the following:

   a. Mycobacteria spp
   b. M. tuberculosis
   c. M. avium intracellulare

4. For patients whose sputum is AFB smear positive or Hologic Amplified MTD positive molecular-based drug susceptibility testing **MEETS COVERAGE CRITERIA** when one of the following is met:
   a. The individual has been treated for tuberculosis in the past
   b. The individual was born in or has or has lived for at least 1 year in a foreign country with at least a moderate TB incidence (≥20 per 100 000) or a high primary multi-drug resistant (MDR)-TB prevalence (≥2%)
   c. The individual is a contact of an individual with MDR-TB
   d. The individual isHIV infected 5. Repeat drug susceptibility testing **MEETS COVERAGE CRITERIA** in the following situations:
   a. For individuals whose h sputum cultures remain positive after 3 months of treatment.
   b. When there is bacteriological reversion from negative to positive.

6. In patients with pleural effusion, pericardial effusion, or ascites and suspected TB infection, cell counts, protein, glucose, and lactate dehydrogenase (LDH) concentrations of cerebrospinal, pleural, peritoneal, pericardial and other fluids **MEETS COVERAGE CRITERIA**.

7. In HIV-infected patients with CD4 cell counts ≤100 cells/microL who have signs and symptoms of tuberculosis, urine-based detection of mycobacterial cell was glycolipid lipoarabinomannan (LAM) **MEETS COVERAGE CRITERIA**.

8. IGRA **DOES NOT MEET COVERAGE CRITERIA** for patients with active tuberculosis.

9. Simultaneous ordering of any combination of direct probe, amplified probe, and/or quantification for the same organism in a single encounter **DOES NOT MEET COVERAGE CRITERIA**.

10. Testing of adenosine deaminase (ADA) and interferon-gamma (IFN- γ) levels in cerebrospinal, pleural, peritoneal, pericardial, and other fluids for the diagnosis of extrapulmonary TB **DOES MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

1. Quantitative nucleic acid testing for *Mycobacterium* spp, *M. tuberculosis*, and *M. avium intracellulare* **DOES NOT MEET COVERAGE CRITERIA**.
2. Whole genome sequencing of *Mycobacterium* spp. for the detection of drug resistance **DOES NOT MEET COVERAGE CRITERIA**.
3. Genotyping of *Mycobacterium* spp **DOES NOT MEET COVERAGE CRITERIA**.
4. Testing of serum protein biomarkers or panels of biomarkers for the detection and diagnosis of TB **DO NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Tuberculosis (TB) continues to be a major public health threat globally, causing an estimated 10.0 million new cases and 1.2 million deaths from TB among HIV-negative individuals and 208,000 deaths among HI-positive people in 2019 (WHO, 2020), with the emergence of multidrug resistant strains only adding to the threat (Dheda et al., 2014). The lungs are the primary site of infection by Mtb and subsequent TB disease. Onset of symptoms is usually gradual with a persistent cough being most frequently reported (95%) followed by the typical symptoms of fever (75%), night sweats (45%) and weight loss (55%) (Heemskerk et al., 2015). Clinical manifestations include primary TB, reactivation TB, laryngeal TB, endobronchial TB, lower lung field TB infection, and tuberculoma (Bernardo, 2022). Extrapulmonary infection represents approximately 20% of cases of active TB with an additional 7% having concurrent pulmonary and extrapulmonary infections (Peto et al., 2009).

In most individuals, initial *Mycobacterium tuberculosis* infection is eliminated, or contained by host defenses, while infection remains latent (Barry et al., 2009; Dheda et al., 2010). Persons with latent TB

infection (LTBI) are considered to be asymptomatic and not infectious; however, latent Mtb bacilli may remain viable and reactivate to cause active, contagious infection. Identification and treatment of LTBI are important TB control strategies, especially in settings with a low TB incidence, where reactivation of LTBI often accounts for the majority of nonimported TB disease (ATS, 2000; Landry & Menzies, 2008; Pai et al., 2014).

Latent TB Testing (LTBI)

The goal of testing for LTBI is to identify individuals who are at increased risk for the development of tuberculosis (TB) and therefore who would benefit from treatment of latent TB infection. Only those who would benefit from treatment should be tested so a decision to test presupposes a decision to treat if the test is positive (Menzies, 2022).

Proprietary Testing

The Bactec MGIT 960 System was approved by the FDA in 1998 for the detection of mycobacteria growth from clinical specimens (except blood).

In 1994 the FDA approved the Ge-Probe Amplified Mycobacterium Tuberculosis Direct Test as a Nucleic acid-based in vitro diagnostic devices for the detection of *Mycobacterium tuberculosis* complex in respiratory specimens. These devices are non-multiplexed and intended to be used as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings.

In 2015 the FDA approved the Xpert® MTB/RIF Assay, performed on the GeneXpert® Instrument Systems, as a qualitative, nested real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of *Mycobacterium tuberculosis* complex DNA in raw sputum or concentrated sputum sediment prepared from induced or expectorated sputum. In specimens where *Mycobacterium tuberculosis* complex (MTB-complex) is detected, the Xpert MTB/RIF Assay also detects the rifampin-resistance associated mutations of the *rpoB* gene.

The QuantiFERON-TB® assay (CSL Biosciences, Australia) for detection of gamma interferon production is a blood test that has been used in humans in Australia. In November 2001, this test received approval from the U.S. Food and Drug Administration (FDA) in the United States for the following indication: "The QuantiFERON-TB test is intended as an aid in the detection of latent Mycobacterium tuberculosis infection."

In December of 2004, QuantiFERON-TB® GOLD received FDA approval for the detection of latent TB. This test differs from the first-generation test in that instead of using PPD as the stimulus for interferon production, 2 antigens, ESAT-6 and CFP-10, are used. These antigens are present in mycobacterium tuberculosis but are not present in those exposed to BCG or non-tuberculous mycobacteria.

The QFT-GIT measures IFN-γ plasma concentration using an enzyme-linked immunosorbent assay (ELISA), has been approved by the US Food and Drug Administration (FDA) and has replaced the QuantiFERON-TB Gold (QFT-G) test (Lewinsohn et al., 2017).

The T-SPOT assay enumerates T cells releasing IFN-γ using an enzyme-linked immunospot (ELISPOT) assay. The T-SPOT.TB assay is currently available in Europe, Canada, and has been approved for use in the United States with revised criteria for test interpretation (Lewinsohn et al., 2017)

Analytical Validity

Mycobacterial infection results in a predominantly cell-mediated immune response (Daniel, 1980). Skin testing (TST) has long been a convenient, cost-effective method for assessing cell-mediated immune responses to a variety of antigens (Snider, 1982) and has been the "gold standard" for diagnostic screening for *Mycobacterium tuberculosis* infections. However, multiple factors challenge the accuracy of the skin test, including skill requirements for and variability in placement and reading, cross-reactivity, and underlying illness or immunosuppression (Daniel, 1980; Snider, 1982). The sensitivity of the TST is approximately 71%–82% (Francis et al., 1978; Katial et al., 2001; Lewinsohn et al., 2017).

The cell-mediated immune response to *M. tuberculosis* involves production of gamma interferon (IFN-γ) (Fenton et al., 1997). Interferon-gamma release assays (IGRAs), which are in-vitro culture assays measuring IFN-γ production in response to tuberculin antigen stimulation, have been developed as diagnostic screening tests (Katial et al., 2001; Lein & Von Reyn, 1997) IGRAs have specificity >95% for diagnosis of latent TB infection and a sensitivity of 80-90% (Menzies et al., 2007; Pai et al., 2014). The two commercially available IGRAs are the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay and T-SPOT.TB assay. Both assays are FDA-approved and available worldwide. These tests are not used to diagnose an active infection (as active infections are microbiologic diagnoses), but they still provide use as a confirmatory test for the TST (Menzies, 2022).

Clinical Utility and Validity

LTBI Testing

Diel et al. (2012) performed a meta-analysis investigating the "positive and the negative predictive value (PPV and NPV, respectively) from a test-determined LTBI state for progression to active TB of interferon-γ release assays (IGRAs) and the tuberculin skin test (TST)". The authors found that the "pooled PPV for progression for all studies using commercial IGRAs was 2.7% compared with 1.5% for the TST." PPV was found to increase to 6.8% and 2.4% respectively when only high-risk groups were included. The authors concluded that "Commercial IGRAs have a higher PPV and NPV for progression to active TB compared with those of the TST(Diel et al., 2012)."

Ruan et al. (2016) further assessed the "diagnostic value of interferon-γ release assays (IGRAs) for latent tuberculosis infection (LTBI) in patients with rheumatic disease before receiving biologic agents." 11 studies (n = 1940) were included. The authors found that "compared with the tuberculin skin test (TST), the pooled agreements in QFT-G/GIT and T-SPOT.TB were 72% and 75%, respectively. BCG vaccination was positively correlated with positive rates of TST (pooled odds ratio [OR] 1.64). Compared with TST, IGRAs were better associated with the presentence of one or more tuberculosis (TB) risk factors." The authors concluded that "in rheumatic patients with previous BCG vaccination or currently on steroid therapy, IGRAs would be the better choice to identify LTBI by decreasing the false-positivity and false-negativity rate compared with conventional TST (Ruan et al., 2016)."

Auguste et al. compared IGRA and TST for identifying latent tuberculosis infection that progresses to active tuberculosis. 17 studies were included. However, no significant differences were observed, and the authors concluded that "prospective studies comparing IGRA testing against TST on the progression from LTBI to TB were sparse, and these results should be interpreted with caution due to uncertainty, risk of bias, and unexplained heterogeneity. Population-based studies with adequate sample size and follow-up are required to adequately compare the performance of IGRA with TST in people at high risk of TB (Auguste et al., 2017)."

Nasiri et al. performed a meta-analysis focusing on the diagnostic accuracy of IGRA and TST for LTBI in transplant patients. 16 articles were included, and the results are as follows: "pooled sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR) for TST were 46%, 86%, 46.3%, 88.7%, 3.3, 0.63, and 5 respectively. For QFT-G (an IGRA), the pooled sensitivity, specificity, PPV, NPV, PLR, NLR, and DOR were 58%, 89%, 72.7%, 80.6%, 5.3, 0.47, and 11, respectively. Likewise, for T-SPOT.TB (another IGRA), the pooled sensitivity, specificity, PPV, NPV, PLR, NLR, and DOR were 55%, 92%, 60.4%, 90.2%, 6.7, 0.52, and 16, respectively". The authors concluded that "IGRAs were more sensitive and specific than the TST with regard to the diagnosis of LTBI in the transplant candidates. They have added value and can be complementary to TST (Nasiri et al., 2019)."

Hacioglu et al. (2022) investigated the "reliability of TST with respect to BCG vaccination and drugs in candidates for TNFi [TNF-alpha inhibitor] treatment."From 1031 patients who were prescribed a TNFi for the first time, they found upon univariate analysis, "male sex, BCG vaccination, sulfasalazine, and diagnosis of ankylosing spondylitis as predictors of positive TST and use of prednisolone, methotrexate, hydroxychloroquine, leflunomide, azathioprine, and diagnosis of rheumatoid arthritis and inflammatory bowel disease as predictors of negative TST for both 5 and 10 mm cutoff values." However, on a multivariate regression, only male sex and BCG vaccination were associated with positive TST for both 5mm and 10mm cutoff values, and azathioprine and prednisolone use were both associated with negative TST at the 5mm cutoff. Azathioprine was still associated with a negative TST at the 10mm cutoff as well. This may be a result of suppressing the TST response with those dosages for inflammatory disease treatment. The researchers concluded that "screening for laten tuberculosis with TST causes more patients to be treated for latent tuberculosis compared to QTF-GT [Quantiferon TB-Gold]."This indicated what methods may be better for diagnosis of tuberculosis to initiate and expedite treatment.

Active TB Testing

The diagnosis of TB disease should be suspected in patients with relevant clinical manifestations and exposure history (Lewinsohn et al., 2017). Laboratory testing is an integral part of the rapid and accurate diagnosis of TB to facilitate timely initiation of treatment.

Microbiologic testing is used to evaluate an active TB infection. These tests may include the acid-fast bacilli smear (AFB), the mycobacterial culture, and molecular testing. Smears are the fastest and cheapest diagnostic tool, cultures are the most sensitive, and molecular testing is used for assessing drug resistance (Bernardo, 2022).

The detection of acid-fast bacilli (AFB) on microscopic examination of stained sputum smears is the most rapid and inexpensive technique (Bernardo, 2022); however, it is limited by its lack of sensitivity in certain situations, such as extrapulmonary infection or coinfection with HIV (Pai et al., 2016). The mycobacteria retain the stain in a mineral-acid or acid-alcohol solution, and microscopy identifies these strains. LED microscopy has seen more use recently than the traditional light microscopy (Bernardo, 2022).

Rapid and accurate diagnosis is critical for timely initiation of TB treatment (Pai et al., 2016). Although sensitive, culture can take over two weeks to return results (Lewinsohn et al., 2017). Three specimens should be examined to assure a sensitivity of approximately 70%. The first specimen has a sensitivity of approximately 53.8%, increasing by 11.1% with a second specimen, and another 2-5% with a third (Mase et al., 2007). A first morning specimen increases sensitivity by 12%, and concentrating specimens can increase sensitivity by 18% (Steingart, Ng, et al., 2006). Use of fluorescence microscopy also increases sensitivity 10% over conventional microscopy (Lewinsohn et al., 2017; Steingart, Henry, et al., 2006). The positive predictive value has been reported to be 97.9-100% (Gordin & Slutkin, 1990), but it is impacted by non-tuberculosis *Mycobacterium* species (NTM) (Yajkoet al., 1994).

Nucleic acid amplification techniques (NAAT) have been developed for rapid diagnosis of TB. Two major tests are available, the Amplified Mycobacterium tuberculosis Direct (MTD) test and the Xpert MTB/RIF test. NAAT-based assays are more sensitive than smear, but less sensitive than culture, with a reported sensitivity of 96% and specificity of 99% (Greco et al., 2006; Lewinsohn et al., 2017). NAAT testing has >95% positive predictive value in the setting of AFB smear-positive specimens for distinguishing tuberculous from nontuberculous mycobacteria, and it can establish the presence of tuberculosis in 50 to 80% of AFB smear-negative specimens (Cheng et al., 2005). NAAT does not replace the roles of AFB smear and culture (Ling et al., 2008) in the diagnostic algorithm for tuberculosis and results must be interpreted in conjunction with AFB smear results while mycobacterial culture is pending (CDC, 2009; Lewinsohn et al., 2017).

Sequence-based assays provide the genetic identity of a particular mutation and, therefore, can predict drug resistance with greater accuracy than probe-based assays. The testing identifies genetic mutations associated with rifampin and isoniazid resistance as well as resistance to second-line drugs including fluoroquinolones and the injectables amikacin, kanamycin, and capreomycin. Molecular testing results are generally available within days and can be used to guide initial treatment decisions and inform design of prevention regimens for contacts (Bernardo, 2022; Taylor et al., 2005).

More proprietary tests exist for the assessment of TB. Rapid Biosensor (RBS) offers a breath test "TB Breathalyzer" for TB. The test proposes that it can detect actively infectious bacilli instead of relying on sputum (which some patients do not produce). The test estimates its limit of detection at 25-75 bacilli and notes that it can be used easily in rural communities. When a patient coughs in the collection tube, any TB bacilli will react with the biochemical formulation at the bottom of the tube, which is then detected by the diode laser in the reader unit (RBS, 2015).

The reference standard for diagnosis of any TB infection is isolation of *M. tuberculosis* (Pai et al., 2016). The isolate recovered should be identified according to the Clinical and Laboratory Standards Institute

guidelines (Institute, 2018) and the American Society for Microbiology Manual of Clinical Microbiology (Lewinsohn et al., 2017; Woods et al., 2015), and all United States jurisdictions require submission of culture isolates identified as *M. tuberculosis* for confirmation of identification and drug susceptibility testing (Taylor et al., 2005). Positive cultures are also reported to public health authorities for oversight and case management (Bernardo, 2022).

Clinical Utility and Validity – Active TB

Cruciani et al. (2004) performed a meta-analysis of 10 studies (1381 strains from 14745 clinical specimens) which found that both liquid and solid culture media methods are highly specific (99%). Liquid culture methods are more sensitive (81.5-85.8%) and have a shorter time to detection (13.2-15.2 day) than solid media but are more prone to contamination (4-9%). Solid media has a sensitivity of 76% and averages 25.8 days for detection. The use of both culture methods increases the overall sensitivity to 87.7-89.7%.

Bourgi et al. "aimed to evaluate the reliability and projected impact of nucleic acid amplification (NAA) testing in patients with acid-fast bacilli (AFB) smear-positive respiratory samples". The authors identified a retrospective cohort of AFB smear-positive patients and evaluated the projected change in "duration of airborne isolation and unnecessary Mycobacterium tuberculosis (MTB) treatment with introducing NAA testing into clinical decision making for AFB smear-positive patients". 130 patients were found to be AFB positive, of which 80 tested positive on NAA. 82 patients grew MTB on culture. NAA testing was found to have a sensitivity of 97.6% and specificity of 100%. Integrating NAA testing into clinical decision making led to shortened time in airborne isolation (6.0 ± 7.6 vs 23.1 ± 38.0) and 9.5 ± 11.32 fewer days of "unnecessary MTB treatment in patients with negative NAA test." The authors concluded, "Nucleic acid amplification testing provided a rapid and accurate test in the diagnosis of MTB while significantly reducing the duration of isolation and unnecessary medications in patients with negative NAA test (Bourgi et al., 2017)."

Urine testing for mycobacterial cell wall glycolipid (Shah et al., 2010) has been investigated as a point of care assay for diagnosis of TB in HIV infected patients (Nakiyingi et al., 2014). The test was 97.6% specific and 67.9% sensitive in patients with CD4<100. It is useful in addition to routine diagnostic tests for HIV-infected patients with signs and symptoms of TB and CD4 ≤100 cells/microL and for all HIV-infected patients who are seriously ill (Shah et al., 2016; WHO, 2015a). Gupta-Wright et al. (2018) evaluated the sputum Xpert MTB/RIF with or without urine lipoarabinomannan (LAM) testing. There was no difference in overall mortality over 2574 patients, but they found that urine Lam testing might benefit some high-risk subgroups (CD4 <100, severe anaemia, and patients with clinically suspected tuberculosis) (Gupta-Wright et al., 2018).

Adenosine deaminase (ADA) and interferon-gamma (IFN- γ) levels in cerebrospinal, pleural, peritoneal, and pericardial fluids have been studied in the diagnosis of extrapulmonary TB. A joint review by the ATS, IDSA, and CDC found the sensitivity of ADA in these fluids to be 79% and the specificity to be 83% for TB. The sensitivity of IFN- γ in these fluids was 89% and the specificity was 97%. However, the authors remarked that neither the ADA level nor the IFN-γ level provide a definitive diagnosis of TB disease (Lewinsohn et al., 2017).

De Groote et al. developed a panel based on proteomic analysis. 1470 serum samples were collected from patients "with symptoms and signs suggestive of active pulmonary TB that were systematically confirmed or ruled out for TB by culture and clinical follow-up". Six protein biomarkers were identified: "SYWC, kallistatin, complement C9, gelsolin, testican-2, and aldolase C", which performed well in a training set (area under curve = 0.92) to distinguish between TB and non-TB. It was also found to have 90% sensitivity and 80 % specificity. The authors concluded that their panel "warrants diagnostic development on a patient-near platform" (De Groote et al., 2017).

Heyckendorf et al. compared the utility of genotypic and phenotypic assays for evaluation of tuberculosis (TB) drug resistance. The authors used the results from the assays to develop treatment regimens for the 25 multi- and extensively drug-resistant tuberculosis patients in the study. Compared to phenotypic assay-developed regimens, whole genome sequencing (WGS) yielded a regimen of drugs at 93% agreement with the phenotypic assay's regimen. Further, the whole genome sequencing-derived regimen did not contain any drugs identified as resistant by the phenotypic assay. However, the authors commented that "MIC [minimum inhibitory concentration] testing revealed that pDST [phenotypic drug susceptibility testing] likely underestimated the true rate of resistance for key drugs (rifampin, levofloxacin, moxifloxacin, and kanamycin) because critical concentrations (CCs) were too high". Results derived from other genotypic assays (Xpert, line probe assays) had lower agreement with the phenotypic assay (49% and 63% respectively). The authors concluded that "WGS can be used to rule in resistance even in M/XDR strains with complex resistance patterns, but pDST for some drugs is still needed to confirm susceptibility and construct the final regimens. Some CCs for pDST need to be reexamined to avoid systematic false-susceptible results in low-level resistant isolates" (Heyckendorf et al., 2018).

Ustinova et al. investigated an assay's ability to identify and distinguish between nontuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis* complex (MTBC) in culture and sputum. 301 NTM cultures with mycobacteriosis were measured, and sputum samples were contributed by "104 patients with mycobacteriosis, 3627 patients with tuberculosis and 118 patients with other lung diseases". The authors results were as follows: "Specificity and sensitivity of the assay for MTBC was found to be 100% both in culture and sputum samples; for NTM, the specificity was 100% in culture and sputum, the sensitivity reached 100% in culture and 73.1% in sputum samples. Positive predictive value (PPV) and negative predictive value (NPV) of the assay for culture were both 100%, for clinical material 100% and 80.8%, respectively" (Ustinova et al., 2019).

Adams et al. compared the performances of the tuberculin skin test (TST) and two interferon-gamma-release assays (IGRAs). Five hundred and five health care workers (HCWs) in Cape Town, South Africa, were screened for latent tuberculosis infection (LTBI) using the three assays. The authors identified LTBI prevalence to be 81%. TST at a cut-off of 10 mm had the highest sensitivity at 93% and the lowest specificity at 57%. The QFT-GIT IGRA sensitivity was 80% and specificity was 96%; the TSPOT.TB IGRA sensitivity was 74% and specificity was 96%. Positive predictive values for IGRAs was 90% and 96% for TST and the highest negative predictive value was 66%. However, a composite rule using both TST and QFT-GIT improved negative predictive value to 90%. The authors concluded that "in an endemic setting

a positive TST or IGRA was highly predictive of LTBI, while a combination of TST and IGRA had high rule-out value (Adams et al., 2019).

Zürcher et al. evaluated the "mortality in patients with tuberculosis from high-burden countries, according to concordance or discordance of results from drug susceptibility testing done locally and in a reference laboratory". A total of 634 patients were included, 272 of which were HIV-positive. The authors identified 394 strains (62%) to be "pan-susceptible", 45 (7%) to be monoresistant, 163 (27%) to be multi-drug resistant, and 30 (5%) to be "extensively" resistant. The laboratory results were concordant for 513 (81%) patients and discordant for 121 (19%) patients, resulting in a 90.8% sensitivity and 84.3% specificity. The authors identified a 7.33 odds ratio of death for patients with discordant results, which potentially led to under-treatment. The authors concluded "inaccurate drug susceptibility testing by comparison with a reference standard leads to under-treatment of drug-resistant tuberculosis and increased mortality" (Zürcher et al., 2019).

Jain et al. (2021) conducted a cross-sectional study in India to "compare the performance of GeneXpert MTB/RIF (GXpert) assay with [the] composite reference standard in diagnosing cases of tubercular pleural effusion (TPE) and to evaluate the reliability of rifampicin resistance." In diagnosing TPE, the sensitivity of the assay was 16.6% among 158 study participants, with a specificity of 100%, diagnostic accuracy of 52.5%, positive predictive value of 100%, and negative predictive value of 47.5%. Because of these findings, the researchers concluded that this GXpert assay would need to be combined with "routine pleural fluid analysis" to accurately diagnose TPE in suspected patients (Jain et al., 2021).

Karthek et al. (2021) evaluated the usage of the same GeneXpert MTB/RIF assay in the context of spinal tuberculosis. In conducting a retrospective review from 136 patients that underwent spinal biopsy for spondylodiscitis, 86 final patients met the criteria for spinal tuberculosis (61.6% demonstrated Mtb positivity in tissue samples and 38.4% were positive through pus samples). From this data, the researchers found a 65.1% sensitivity, 100% specificity, 100% PPV, and 56.5% NPV for this assay. It was also accurate in detecting drug resistance among patient specimen (Karthek et al., 2021).

V. Guidelines and Recommendations

World Health Organization (WHO)

- The WHO published recommendations for the diagnosis of TB which state:
- Mycobacteria can be visually distinguished from other microorganisms by their thick lipid containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (known as 'acid fastness'). Given that the examination of two sputum specimens is adequate to identify the majority (95-98%) of smear-positive TB patients, WHO's current policy on case-finding using microscopy recommends that in settings with appropriate external quality assessment and documented good-quality microscopy two specimens should be examined (WHO, 2015b).
- "Direct Ziehl–Neelsen staining of sputum specimens and examination using light microscopy is suitable for use at all levels of laboratory, including peripheral laboratories at primary health-care centres or district hospitals. There is insufficient evidence that processed sputum specimens (for example, those that are concentrated or chemically treated) give better results than direct smear microscopy. Therefore, the use of such methods is not recommended" (WHO, 2015b).

- "Evidence shows that the diagnostic accuracy of LED microscopy is comparable to that of conventional fluorescence microscopy and it surpasses that of conventional Ziehl–Neelsen microscopy (by an average of 10%). Therefore, WHO recommends replacing conventional fluorescence microscopy with LED microscopy, and that LED microscopy should be phased in as an alternative to conventional Ziehl–Neelsen light microscopy in all settings, prioritizing high-volume laboratories" (WHO, 2015b).
- "Mycobacteria can be cultured in specific solid or liquid media. Bacterial growth can be identified visually (that is, by identifying specific characteristics) or by automated detection of its metabolism. All positive mycobacterial cultures must be tested to confirm the identification of *M. tuberculosis* complex (MTBC)" (WHO, 2015b).
- "Differentiation of the members of the MTBC is necessary for the treatment of individual patients and for epidemiological purposes, especially in areas of the world where tuberculosis has reached epidemic proportions or wherever the transmission of M. bovis between animals or animal products and humans is a problem. In addition, it can be important to rapidly identify isolates of M. bovis bacillus Calmette-Guérin (BCG) recovered from immunocompromised patients. Differentiation of species with the MTBC can be achieved using either phenotypic26 and/ or genotypic methods" (WHO, 2015b).
- "The use of rapid immunochromatographic assays (or strip tests for speciation) to identify cultured isolates is recommended because they provide definitive identification of all members of the MTBC (including M. bovis) in 15 minutes" (WHO, 2015b).
- "WHO recommends that either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100000 population" (WHO, 2015a).
- "It is strongly recommended that commercial serodiagnostic tests not be used for the diagnosis of pulmonary and extra-pulmonary TB. Currently available commercial serodiagnostic tests (also referred to as serological tests) provide inconsistent and imprecise findings. There is no evidence that existing commercial serological assays improve patient outcomes, and high proportions of false positive and false-negative results may have an adverse impact on the health of patients" (WHO, 2015b).
- "There is no consistent evidence that IGRAs are more sensitive than TST for diagnosis of active TB disease. Studies evaluating the incremental value of IGRAs to conventional microbiological tests show no meaningful contribution of IGRAs to the diagnosis of active TB. IGRAs are considered inadequate as rule-out or rule-in tests for active TB, especially in the context of HIV infection. IGRAs should not be used for the diagnosis of active TB disease" (WHO, 2015b).

The following recommendations involve LTBI (WHO, 2018).

- "Either a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) can be used to test for LTBI."
- "LTBI testing by TST or IGRA is not a requirement for initiating preventive treatment in people living with HIV or child household contacts aged < 5 years. (Strong recommendation, moderate-quality evidence. Updated recommendation)"

- "Adults and adolescents living with HIV should be screened for TB according to a clinical algorithm. Those who do not report any of the symptoms of current cough, fever, weight loss or night sweats are unlikely to have active TB and should be offered preventive treatment, regardless of their ART status."
- "People living with HIV who have a positive test for LTBI benefit more from preventive treatment than those who have a negative LTBI test; LTBI testing can be used, where feasible, to identify such individuals."
- "Patients initiating anti-TNF treatment, patients receiving dialysis, patients preparing for an organ or haematological transplant and patients with silicosis should be systematically tested and treated for LTBI. (Strong recommendation, low–very low-quality evidence. Updated recommendation)"
- "In countries with a low TB incidence, systematic testing for and treatment of LTBI may be considered for prisoners, health workers, immigrants from countries with a high TB burden, homeless people and people who use illicit drugs. (Conditional recommendation, low–very low-quality evidence. Existing recommendation)"
- "Systematic testing for LTBI is not recommended for people with diabetes, people with harmful alcohol use, tobacco smokers and underweight people unless they are already included in the above recommendations. (Conditional recommendation, very low-quality evidence. Existing recommendation)"
- "There is no gold standard method for diagnosing LTBI. TST and IGRA require a competent immune response in order to identify people infected with TB and are imperfect tests for measuring progression to active disease." (WHO, 2018)

The WHO also published an additional guideline in 2020, which discusses preventive treatment. Some relevant recommendations and comments are listed below:

- "Either a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) can be used to test for LTBI."
- "There is no strong evidence that one test should be preferred over the other in terms of predicting progression from TB infection to TB disease. Neither TSTs nor IGRAs should be used in persons having a low risk of TB infection and disease."
- A testing algorithm was also published in the guideline, which discusses latent TB testing and subsequent treatment in individuals at risk. The guideline writes that both asymptomatic household contacts (of patients with TB), as well as members of non-HIV risk groups (such as patients with "silicosis, dialysis, anti-TNF agent treatment, preparation for transplantation or other risks in national guidelines" should be tested with TST or IGRA.
- "There is no gold standard method for diagnosing LTBI. TST and IGRA require a competent immune response in order to identify people infected with TB and are imperfect tests for measuring progression to active disease."

Finally, the WHO published an extensive guideline on the diagnosis of tuberculosis. Some relevant recommendations and comments are listed below:

- "In adults with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and rifampicin-resistance detection in sputum rather than smear microscopy/culture and phenotypic DST. (Strong recommendation, high certainty of evidence for test accuracy; moderate certainty of evidence for patient-important outcomes)"
- "In children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and rifampicin-resistance detection in sputum, gastric aspirate, nasopharyngeal aspirate and stool rather than smear microscopy/culture and phenotypic DST. (Strong recommendation, moderate certainty for accuracy in sputum; low certainty of evidence for test accuracy in gastric aspirate, nasopharyngeal aspirate and stool)"
- "In adults with signs and symptoms of pulmonary TB and without a prior history of TB (≤5 years) or with a remote history of TB treatment (>5 years since end of treatment), Xpert Ultra should be used as an initial diagnostic test for TB and for rifampicin-resistance detection in sputum, rather than smear microscopy/culture and phenotypic DST. (Strong recommendation, high certainty of evidence for test accuracy)"
- "In adults with signs and symptoms of pulmonary TB and with a prior history of TB and an end of treatment within the last 5 years, Xpert Ultra may be used as an initial diagnostic test for TB and for rifampicin-resistance detection in sputum, rather than smear microscopy/culture and phenotypic DST. (Conditional recommendation, low certainty of evidence for test accuracy)"
- "In children with signs and symptoms of pulmonary TB, Xpert Ultra should be used as the initial diagnostic test for TB and detection of rifampicin resistance in sputum or nasopharyngeal aspirate, rather than smear microscopy/culture and phenotypic DST. (Strong recommendation, low certainty of evidence for test accuracy in sputum; very low certainty of evidence for test accuracy in nasopharyngeal aspirate)"
- "In adults and children with signs and symptoms of TB meningitis, Xpert MTB/RIF or Xpert Ultra should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for TB meningitis rather than smear microscopy/culture. (Strong recommendation, moderate certainty of evidence for test accuracy for Xpert MTB/RIF; low certainty of evidence for test accuracy for Xpert Ultra)"
- "In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF may be used in lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine specimens as the initial diagnostic test for respective form of extrapulmonary TB rather than smear microscopy/culture. (Conditional recommendation, moderate certainty of evidence for test accuracy for pleural fluid; low certainty for lymph node aspirate, peritoneal fluid, synovial fluid, urine; very low certainty for pericardial fluid, lymph nodes biopsy)"
- "In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra may be used in lymph node aspirate and lymph node biopsy as the initial diagnostic test for lymph nodes TB rather than smear microscopy/culture. (Conditional recommendation, low certainty of evidence)"
- "In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF or Xpert Ultra should be used for rifampicin-resistance detection rather than culture and phenotypic DST. (Strong recommendation, high certainty of evidence for test accuracy for Xpert MTB/RIF; low certainty of evidence for Xpert Ultra)"

- "In HIV-positive adults and children with signs and symptoms of disseminated TB, Xpert MTB/RIF may be used in blood, as an initial diagnostic test for disseminated TB. (Conditional recommendation, very low certainty of evidence for test accuracy)"
- "In adults with signs and symptoms of pulmonary TB who have an Xpert Ultra trace positive result on the initial test, repeated testing with Xpert Ultra may not be used."
- "In children with signs and symptoms of pulmonary TB in settings with pretest probability below 5% and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF in sputum, gastric fluid, nasopharyngeal aspirate or stool specimens may not be used."
- "In children with signs and symptoms of pulmonary TB in settings with pretest probability 5% or more and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF (for total of two tests) in sputum, gastric fluid, nasopharyngeal aspirate and stool specimens may be used."
- "In children with signs and symptoms of pulmonary TB in settings with pretest probability below 5% and an Xpert Ultra negative result on the initial test, repeated testing with Xpert Ultra in sputum or nasopharyngeal aspirate specimens may not be used."
- "In children with signs and symptoms of pulmonary TB in settings with pretest probability 5% or more and an Xpert Ultra negative result on the first initial test, repeated one Xpert Ultra test (for a total of two tests) in sputum and nasopharyngeal aspirate specimens may be used."
- "In adults in the general population who had either signs or symptoms of TB or chest radiograph with lung abnormalities or both, the Xpert MTB/RIF or Xpert Ultra may replace culture as the initial test for pulmonary TB. (Conditional recommendation, low certainty of the evidence in test accuracy for Xpert MTB/RIF and moderate certainty for Xpert Ultra)"
- "In adults and children with signs and symptoms of pulmonary TB, the Truenat MTB or MTB Plus may be used as an initial diagnostic test for TB rather than smear microscopy/culture. (Conditional recommendation, moderate certainty of evidence for test accuracy)"
- "In adults and children with signs and symptoms of pulmonary TB and a Truenat MTB or MTB Plus positive result, Truenat MTB-RIF Dx may be used as an initial test for rifampicin resistance rather than culture and phenotypic DST. (Conditional recommendation, very low certainty of evidence for test accuracy)"
- "For persons with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs [line probe assays] may be used as the initial test instead of phenotypic culture-based DST to detect resistance to rifampicin and isoniazid."
- "LPAs are not recommended for the direct testing of sputum smear-negative specimens."
- "These recommendations do not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance."
- "Conventional culture-based DST for isoniazid may still be used to evaluate patients when the LPA result does not detect isoniazid resistance. This is particularly important for populations with a high pretest probability of resistance to isoniazid."

- "For patients with confirmed MDR- or rifampicin-resistant TB (MDR/RR-TB), second-line LPAs are recommended to detect additional resistance to second-line anti-TB agents."
- "WHO suggests using LF-LAM [Lateral flow urine lipoarabinomannan assay] to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children: with signs and symptoms of TB (pulmonary and/or extrapulmonary) or seriously ill (conditional recommendation, low certainty in the evidence about test accuracy); and irrespective of signs and symptoms of TB and with a CD4 cell count of less than 100 cells/mm^3 (conditional recommendation, very low certainty in the evidence about test accuracy)
- "WHO recommends against using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children [in the following settings]: without assessing TB symptoms, without TB symptoms and unknown CD4 cell count or without TB symptoms and CD4 cell count greater than or equal to 200 cells/mm^3, or without TB symptoms and with a CD4 cell count of 100–200 cells/mm^3.
- Finally, the WHO did not discuss who genome sequencing of clinical isolates in the context of assessing drug resistance susceptibility for TB. (WHO, 2020a)

American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention

The ATS/IDSA/CDC published clinical practice guidelines for diagnosis of TB in 2017 that stated the following:

LTBI:

- "We recommend performing an interferon-γ release assay (IGRA) rather than a tuberculin skin test (TST) in individuals 5 years or older who meet the following criteria: (1) are likely to be infected with *Mtb*, (2) have a low or intermediate risk of disease progression, (3) it has been decided that testing for LTBI is warranted, and (4) either have a history of BCG vaccination or are unlikely to return to have their TST read (*strong recommendation, moderate-quality evidence*)."
- "We suggest performing an IGRA rather than a TST in all other individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted (*conditional recommendation, moderate-quality evidence*)."
- "There are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been determined that diagnostic testing for LTBI is warranted."
- "Guidelines recommend that persons at low risk for *Mtb* infection and disease progression NOT be tested for *Mtb* infection. We concur with this recommendation. However, we also recognize that such testing may be obliged by law or credentialing bodies. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with *Mtb* despite guidelines to the contrary:"

  - "We suggest performing an IGRA instead of a TST in indivduals 5 years or older (*conditional recommendation, low-quality evidence*). Remarks: A TST is an acceptable alternative in settings where an IGRA is unavailable, too costly, or too burdensome."
  - "We suggest a second diagnostic test if the initial test is positive in individuals 5 years or older (*conditional recommendation, very low-quality evidence*). Remarks: The confirmatory test may be either an IGRA or a TST. When such testing is performed, the person is considered infected only if both tests are positive."

- "We suggest performing a TST rather than an IGRA in healthy children <5 years of age for whom it has been decided that diagnostic testing for LTBI is warranted (*conditional recommendation, very low-quality evidence*)."
- "While both IGRA and TST testing provide evidence for infection with Mtb, they cannot distinguish active from latent TB. Therefore, the diagnosis of active TB must be excluded prior to embarking on treatment for LTBI. This is typically done by determining whether or not symptoms suggestive of TB disease are present, performing a chest radiograph and, if radiographic signs of active TB (eg, airspace opacities, pleural effusions, cavities, or changes on serial radiographs) are seen, then sampling is performed, and the patient managed accordingly."

<u>TB Disease:</u>

- "We recommend that acid-fast bacilli (AFB) smear microscopy be performed, rather than no AFB smear microscopy, in all patients suspected of having pulmonary TB."
- "We suggest that both liquid and solid mycobacterial cultures be performed, rather than either culture method alone, for every specimen obtained from an individual with suspected TB disease."
- "We suggest performing a diagnostic nucleic acid amplification test (NAAT), rather than not performing a NAAT, on the initial respiratory specimen from patients suspected of having pulmonary TB."
- "We recommend performing rapid molecular drug susceptibility testing for rifampin with or without isoniazid using the respiratory specimens of persons who are either AFB smear positive or Hologic Amplified MTD positive and who meet one of the following criteria: (1) have been treated for tuberculosis in the past, (2) were born in or have lived for at least 1 year in a foreign country with at least a moderate tuberculosis incidence (≥20 per 100000) or a high primary multidrug-resistant tuberculosis prevalence (≥2%), (3) are contacts of patients with multidrug-resistant tuberculosis, or (4) are HIV infected."
- "We suggest mycobacterial culture of respiratory specimens for all children suspected of having pulmonary TB."
- "We suggest that cell counts, and chemistries be performed on amenable fluid specimens collected from sites of suspected extrapulmonary TB.
- "We suggest that adenosine deaminase levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB, TB meningitis, peritoneal TB, or pericardial TB."
- "We suggest that free IFN-γ levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB or peritoneal TB."

- "We suggest that AFB smear microscopy be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB.
- "We recommend that mycobacterial cultures be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB."
- "We suggest that NAAT be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB."
- "We suggest that histological examination be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB."
- "Recently, whole-genome sequencing (WGS) has been applied to investigation of tuberculosis outbreaks. This technique may add discriminatory power to strain identification, but the role of WGS in outbreak investigation is still being determined."
- "We recommend one culture isolate from each mycobacterial culture-positive patient be submitted to a regional genotyping laboratory for genotyping (Lewinsohn et al., 2017)."

National Institute of Health (NIH)

The NIH published a set of guidelines regarding opportunistic infections in HIV-positive patients. The NIH writes that "All persons with HIV should be tested for LTBI [latent TB infection] at the time of HIV diagnosis, regardless of their epidemiological risk of TB exposure".

The NIH also comments on diagnostic testing, stating that "sputum smear, nucleic acid amplification (NAA) testing, and culture should be performed in people with HIV with symptoms of TB disease who have a normal chest radiograph, as well as in persons with no pulmonary symptoms but evidence of TB disease elsewhere in the body". The NIH remarks that "pleural fluid, pericardial fluid, ascites, and cerebrospinal fluid should be sampled if there is clinical evidence of involvement exists."

The NIH also discusses drug resistance testing, recommending that "Drug resistance should be considered in all people with HIV, especially those who meet any of the following criteria:

- Known exposure to a person with drug-resistant TB
- Residence in a setting with high rates of primary drug-resistant TB
- Persistently positive smear or culture results at or after 4 months of treatment, or
- Previous TB treatment, particularly if it was not directly observed or was interrupted for any reason."

The NIH recommends "rapid molecular DST for isoniazid and rifampin should be performed on the initial isolates from all patients suspected of having TB, because resistance to isoniazid or rifampin is associated with an increased risk of treatment failure, recurrent TB, and amplification of resistance to additional TB medications."

Overall, the NIH recommends that "For all patients with TB disease, drug-susceptibility [DST] testing to first-line TB drugs (isoniazid, rifampin, ethambutol, and pyrazinamide) should be performed, regardless of the source of the specimen. Molecular resistance testing should be performed, and DSTs should be repeated if sputum cultures remain positive for *M. tuberculosis* at or after 4 months of treatment or

become positive 1 month or longer after culture conversion to negative. Resistance testing for second-line TB medications (fluoroquinolones, bedaquiline, linezolid, clofazimine, aminoglycosides, ethionamide, and others) should be limited to specimens with resistance to first-line TB medications and should be performed in reference laboratories with substantial experience in these techniques." The NIH makes a further stipulation that "isolates with an initial reading of rifampin by commercial NAA test should undergo confirmatory testing (*rpoB* gene sequencing and phenotypic DST). Clinicians who suspect drug-resistant TB in a patient with HIV should make every effort to expedite a diagnosis and consult with their state TB program and then the CDC as needed" (NIH, 2022).

American Thoracic Society, U.S. Centers for Disease Control and Prevention, European Respiratory Society, and Infectious Diseases Society of America (ATS/CDC/ERS/IDSA)

This joint guideline was published to discuss the treatment of drug-resistant tuberculosis. The guideline notes that "molecular DSTs [drug susceptibility tests] should be obtained for rapid detection of mutations associated with resistance. When rifampin resistance is detected, additional DST should be performed immediately for first-line drugs, fluoroquinolones, and aminoglycosides.". The guideline further stated that "A rapid test for a [sic] least rifampin resistance should ideally be done for every patient, but especially for those at risk of drug resistance." Individuals who "have or recently had close contact with a patient with infectious DR-TB [drug resistant tuberculosis] especially when the contact is a young child or has HIV infection, are at risk of developing DR-TB".

The guideline also remarks that if "sputum cultures remain positive after 3 months of treatment, or if there is bacteriological reversion from negative to positive at any time, DST [drug susceptibility testing] should be repeated" and that monthly cultures help to "identify early evidence of failure". Finally, this guideline refers to the above 2017 Lewinsohn guideline as providing "additional details on the optimal use of diagnostic tools and algorithms". (Nahid et al., 2019)

United State Preventative Service Task Force

The USPSTF published a recommendation (2016) which found adequate evidence that accurate screening tests for LTBI are available, treatment of LTBI provides a moderate health benefit in preventing progression to active disease, and the harms of screening and treatment are small. The USPSTF has moderate certainty that screening for LTBI in persons at increased risk for infection provides a moderate net benefit. (Bibbins-Domingo et al., 2016)

Infectious Diseases Society of America (IDSA)/American Society of Microbiology (ASM)

In the 2018 update to the IDSA/ASM joint guideline, A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases, concerning Mycobacterium tuberculosis, they recommend AFB smear or AFB culture when performing laboratory diagnosis. They do allow for the use of NAAT for diagnosing M. tuberculosis; however, they state, "A negative result does not rule out Mycobacterium tuberculosis." They also note that currently there is no commercially available, FDA-approved NAAT for mycobacteria for nonrespiratory samples.

In cases of laboratory diagnosis of pulmonary infections in cystic fibrosis due to suspected *Mycobacterium* spp, they recommend performing a mycobacterial culture from the expectorated sputum, bronchoscopically obtained cultures, or other respiratory cultures. (Miller et al., 2018)

Committee on Infectious Diseases, American Academy of Pediatrics (AAP), 32nd Edition (2021-2024, Red Book)

Highlights from the updated Red Book include the following:

- The AAP notes two NAATs cleared by the FDA for detection of *M. tuberculosis*. Xpert MTB-RIF is considered more sensitive than microscopy but not as sensitive as culture. The AAP also remarks that the CDC recommends a NAAT on at least 1 respiratory tract specimen when TB is suspected.
- For children younger than 2 years, the TST is the preferred method for detection of infection.
- Universal testing with either TST or IGRA is discouraged.
- All organ transplant candidates should be given a TST or IGRA before starting immunosuppression.
- The AAP recommends the following for an "immediate" TST or IGRA:
  - children with suspected TB contact
  - children with clinical or radiographic findings suggesting TB
  - children immigrating from countries with endemic infection
  - children with history of significant travel to countries with endemic infection
- The AAP also recommends an annual TST/IGRA for children with HIV (AAP, 2021).

Tuberculosis Network European Trials Group (TBNET)/RESIST-TB

This consensus statement encompasses molecular drug resistance testing for *Mycobacterium tuberculosis*.

- "Although they do not cover all mutations involved in RMP resistance, molecular methods for RMP could be considered a standard for the diagnostic evaluation of patients with presumptive MDR-TB. In low MDR-TB prevalence countries, physicians should be aware of possible false-positive resistance results of molecular tests, and RMP resistance should be confirmed by a second molecular test on a different sample or by phenotypic tests."
- "Although >90% of RMP-resistant strains are also resistant to INH, molecular testing for INH drug resistance is important."
- "In all patients with evidence of M. tuberculosis with an rpoB mutation in a direct specimen or when DST indicates MDR-TB, molecular testing for second-line resistance should be undertaken to guide treatment and to reduce the time to diagnose XDR-TB."
- "WGS [whole genome sequencing] provides the complete sequence information of the bacterial genome. However, due to the lack of correlation with in vitro (phenotypic DST) and in vivo (treatment outcome) data at present, it is not possible to interpret the clinical value of the vast majority of mutations or polymorphisms detected."

- "The level of discordance between molecular and culture-based DST depends on the drug and the genomic region evaluated. Despite the fact that results of phenotypic methods do not always correspond to response to clinical treatment, culture-based methods are still regarded by most experts involved in this document as the gold standard for DST" (Domínguez et al., 2016).

National Institute for Health and Care Excellence (NICE)

NICE has published guidelines for assessment of TB, which include the following recommendations:

- "If the Mantoux test is positive but a diagnosis of active TB is excluded, consider an interferon gamma release assay if more evidence of infection is needed to decide on treatment."
- "For adults who are severely immunocompromised, such as those with HIV and CD4 counts of fewer than 200 cells/mm^3, or after solid organ or allogeneic stem cell transplant, offer an interferon-gamma release assay and a concurrent Mantoux test."
- "For other adults who are immunocompromised, consider an interferon-gamma release assay alone or an interferon-gamma release assay with a concurrent Mantoux test. If either test is positive (for Mantoux, this is an induration of 5 mm or larger, regardless of BCG history), assess for active TB."
- "Only consider using interferon-gamma release assays alone in children and young people if Mantoux testing is not available or is impractical."
- "If TB is a possibility, microbiology staff should consider carrying out TB culture on samples, even if it is not requested."
- "Request rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum*) on primary specimens if there is clinical suspicion of TB disease, and:
  - the person has HIV or
  - rapid information about mycobacterial species would alter the person's care or
  - the need for a large contact-tracing initiative is being explored."
- "For people with clinically suspected TB, a TB specialist should request rapid diagnostic nucleic acid amplification tests for rifampicin resistance on primary specimens if a risk assessment for multidrug resistance identifies any of the following risk factors:
  - "history of previous TB drug treatment, particularly if there was known to be poor adherence to that treatment"
  - "contact with a known case of multidrug-resistant TB"
  - "birth or residence in a country in which the World Health Organization reports that a high proportion (5% or more) of new TB cases are multidrug-resistant".
- If the rapid diagnostic nucleic acid amplification test for the *M. tuberculosis* complex is negative in a person at high risk of multidrug-resistant TB:
  - "obtain further specimens for nucleic acid amplification testing and culture, if possible"
  - "use rapid rifampicin resistance detection on cultures that become positive for the *M. tuberculosis* complex"

- "If the rapid diagnostic nucleic acid amplification test for rifampicin resistance is positive:
  - "test for resistance to second-line drugs." (NICE, 2019)

European Respiratory Society (ERS) and the European Centre for Disease Prevention and Control (ECDC) Statement: European Union Standards for Tuberculosis Care

This joint guideline was intended to "define the essential level of care for managing patients who have or are presumed to have TB, or are at increased risk of developing the disease."

- "All patients (adults, adolescents and children who are capable of producing sputum) thought to have pulmonary tuberculosis should have at least two sputum specimens submitted for microscopic examination and one for rapid testing for the identification of tuberculosis and drug resistance using an internationally recommended (rapid) molecular test. The sample should be sent for liquid culture and, if positive, for culture-based drug susceptibility testing (DST) in a quality-assured laboratory."
- "For all patients (adults, adolescents and children) presumed to have extrapulmonary tuberculosis, appropriate specimens from the suspected sites of involvement should be obtained for microbiological testing (microscopy, rapid molecular tests, culture, species identification, DST with rapid molecular tests and culture-based techniques) and histopathological examination in quality-assured laboratories."
- "All persons with chest radiographic findings suggestive of pulmonary tuberculosis should have sputum specimens submitted for microscopic examination, rapid molecular tests, culture, species identification and DST with rapid molecular tests and culture-based techniques in a quality-assured laboratory" (ERS/ECDC, 2017).

National Society of Tuberculosis Clinicians (NSTC) of the National Tuberculosis Controllers Association (NTCA)

In February 2021, the NSTC of the NTCA jointly released a set of clinical recommendations for "Testing and Treatment of Latent Tuberculosis Infection in the United States." In relation to testing, the NTSC/NTCA states that "IGRAs are generally preferred, but the TST is acceptable… In choosing which test to use, consider the patient's history of BCG, age, and ability to return for a second appointment. IGRAs offer greater specificity than a TST in persons who were BCG vaccinated or who have non-tuberculous mycobacterial infections. For this reason, IGRAs are preferred for most non-US-born patients who received, or may have received, BCG vaccination. For other persons, either a TST or IGRA can be used depending on test availability and cost."

When discussing immunocompromised patients, the organizations stated that "dual testing with TST and an IGRA simultaneously increases the overall specificity for infection." However, "Dual testing should not be routine, but it may be considered for patients when there is concern about their ability to mount a strong immune response to a test, for persons who are at risk of severe forms of TB disease, or for persons in whom TB infection is strongly suspected because of exposure risks or symptomatology. Children aged <2 years old can be included in a dual testing strategy if one of the above circumstances is present."

With regards to serial testing, "When serial or periodic testing is required, as with some health care personnel at ongoing risk for TB exposure, either an IGRA or the TST may be used. For TST testing, the initial test should be a two-step TST. Because IGRAs do not cause boosting, serial testing with IGRAs does not require two-step testing to establish a baseline."
For persons who are "at low risk for TB infection or active TB disease are required to be tested by law for other reasons, use either an IGRA or TST. If the result is positive, perform a second test with the same or a different method to confirm the test result."
When an MMR vaccine and TB test are both indicated, the Advisory Committee on Immunization Practices recommends:

- "Administer the TST or IGRA simultaneously with the live vaccine (preferred scenario).
- If a TST or IGRA has already been administered, a live vaccine can be administered at any time >1 day after the administration of the TB test.
- If a live vaccine has already been administered, wait at least 28 days before administering a TST or IGRA.
- In two-step testing, wait at least 28 days after the live vaccine is administered before administering the first TST. Continue from there to complete the two-step testing. Wait to administer any additional doses of live vaccine until after the second TST is measured."

In terms of officially diagnosing latent tuberculosis infection, the NSTC states, "At the completion of pretreatment clinical evaluation, if a patient with a positive test result for TB infection does not have any symptoms of TB, and the CXRs and other diagnostic tests results are normal, then active TB disease is excluded and LTBI is diagnosed" (NSTC, 2021).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82945	Glucose, body fluid, other than blood
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83615	Lactate dehydrogenase (LD), (LDH)
84157	Protein, total, except by refractometry; other source (e.g., synovial fluid, cerebrospinal fluid)
84311	Spectrophotometry, analyte not elsewhere specified
86480	Tuberculosis test, cell mediated immunity antigen response measurement; gamma interferon

86481	Tuberculosis test, cell mediated immunity antigen response measurement; enumeration of gamma interferon-producing T-cells in cell suspension
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87116	Culture, tubercle or other acid-fast bacilli (e.g., TB, AFB, mycobacteria) any source, with isolation and presumptive identification of isolates
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87153	Culture, typing; identification by nucleic acid sequencing method, each isolate (e.g., sequencing of the 16S rRNA gene)
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (e.g., antibiotic gradient strip)
87184	Susceptibility studies, antimicrobial agent; disk method, per plate (12 or fewer agents)
87185	Susceptibility studies, antimicrobial agent; enzyme detection (e.g., beta lactamase), per enzyme
87186	Susceptibility studies, antimicrobial agent; microdilution or agar dilution (minimum inhibitory concentration [MIC] or breakpoint), each multi-antimicrobial, per plate
87187	Susceptibility studies, antimicrobial agent; microdilution or agar dilution, minimum lethal concentration (MLC), each plate (list separately in addition to code for primary procedure)
87188	Susceptibility studies, antimicrobial agent; macrobroth dilution method, each agent
87190	Susceptibility studies, antimicrobial agent; mycobacteria, proportion method, each agent
87206	Smear, primary source with interpretation; fluorescent and/or acid fast stain for bacteria, fungi, parasites, viruses or cell types
87550	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria species, direct probe technique
87551	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria species, amplified probe technique
87552	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria species, quantification
87555	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, direct probe technique
87556	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, amplified probe technique
87557	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria tuberculosis, quantification

87560	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria avium-intracellulare, direct probe technique
87561	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria avium-intracellulare, amplified probe technique
87562	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria avium-intracellulare, quantification

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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Testing for Vector-Borne Infections

Policy #: AHS – G2158	**Prior Policy Name & Number (as applicable):** Portions of this policy replace portions of M2097- Identification of Microorganisms using Nucleic Acid Probes; previously titled G2158- Testing Mosquito- or Tick-Related Infections
Implementation Date: 9/15/21	**Date of Last Revision:** 3/22/23; 10/27/23 (see Section VIII)

3.

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Arthropod vectors, including mosquitoes, ticks, fleas, and mites, that feed on vertebrate hosts can spread bacteria, protozoa, and viruses during feeding to their susceptible host, resulting in a variety of infections and diseases. Arboviruses (arthropod-borne viruses) include Zika virus, West Nile virus (WNV), chikungunya virus, dengue virus (DENV), yellow fever virus (YFV), and Colorado tick fever virus (CTF) to name a few. Malaria and babesiosis are both conditions caused by arthropod-borne protozoan parasites, Plasmodium and Babesia, respectively. Conditions caused by arthropod-borne bacteria include rickettsial diseases, ehrlichiosis, anaplasmosis, and Lyme disease, as well as other Borrelia-associated disorders (Calisher, 1994; CDC, 2022a). Isolation, identification, and characterization of these various infections depend on the causative agent. Identification methods may include culture testing, microscopy, and staining techniques; moreover, molecular testing, such as nucleic acid amplification testing (NAAT), and serologic testing, including immunofluorescence antibody assays and enzyme-linked immunosorbent assays (ELISA), can be used for laboratory diagnosis (Miller et al., 2018).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1) For individuals suspected of having babesiosis (see Note 1), the use of a Giemsa- or Wright-stain of a blood smear or NAAT **MEETS COVERAGE CRITERIA.**

2) For individuals suspected of having babesiosis (see Note 1), the use of either an IgG or IgM indirect immunofluorescence antibody (IFA) assay for Babesia **DOES NOT MEET COVERAGE CRITERIA.**

3) For individuals suspected of having chikungunya virus (see Note 2), the use of viral culture for diagnosis, NAAT for the presence of chikungunya in a serum sample, **or** IFA assay for IgM antibodies during both the acute and convalescent phases **MEETS COVERAGE CRITERIA.**

4) For individuals suspected of having Colorado tick fever (CTF) (see Note 3), the use of virus-specific IFA-stained blood smears **or** IFA for CTF-specific antibodies **MEETS COVERAGE CRITERIA.**

5) For the detection of dengue virus (DENV), the use of NAAT, IgM antibody capture ELISA (MAC-ELISA), **or** NS1 ELISA, as well as a confirmatory plaque reduction neutralization test for DENV, **MEETS COVERAGE CRITERIA** in the following individuals:

    a) For individuals suspected of having DENV (see Note 4).

    b) For non-pregnant individuals who are symptomatic for Zika virus infection (see Note 5).

6) For individuals suspected of having DENV (see Note 4), the use of IgG ELISA **or** hemagglutination testing **DOES NOT MEET COVERAGE CRITERIA.**

7) For individuals suspected of having ehrlichiosis and/or anaplasmosis (see Note 6), the use of NAAT of whole blood, IFA assay for IgG antibodies, or microscopy for morulae detection **MEETS COVERAGE CRITERIA**.

8) For individuals suspected of having ehrlichiosis and/or anaplasmosis (see Note 6), the use of an IFA assay for IgM antibodies or standard blood culture **DOES NOT MEET COVERAGE CRITERIA.**

9) For individuals suspected of having malaria (see Note 7), the use of a rapid immunochromatographic diagnostic test or smear microscopy to diagnose malaria, determine the species of Plasmodium, identify the parasitic life-cycle stage, and/or quantify the parasitemia (can be repeated up to three times within three days if initial microscopy is negative in suspected cases of malaria) **MEETS COVERAGE CRITERIA.**

10) For individuals suspected of having malaria (see Note 7), the use of NAAT or IFA for *Plasmodium* antibodies **DOES NOT MEET COVERAGE CRITERIA.**

11) For individuals suspected of having a rickettsial disease (see Note 8), the use of an IFA assay for IgG antibodies (limited to two units) **MEETS COVERAGE CRITERIA.**

12) For individuals suspected of having a rickettsial disease (see Note 8), the use of standard blood culture, nucleic acid amplification testing (NAAT), or IFA assay for IgM antibodies **DOES NOT MEET COVERAGE CRITERIA.**

13) For individuals suspected of having a tick-borne relapsing fever (TBRF) (see Note 9), the use of dark-field microscopy of a peripheral blood smear, microscopy of a Wright- or Giemsa-stained blood smear, PCR testing, or serologic assays to detect *Borrelia* specific IgG antibodies **MEETS COVERAGE CRITERIA**.

14) For individuals suspected of having a TBRF (see Note 9), the use of an IFA assay for IgM for *Borrelia* or culture testing for *Borrelia* **DOES NOT MEET COVERAGE CRITERIA.**

15) For individuals suspected of having West Nile virus (WNV) (see Note 10), the use of IFA for WNV-specific IgM antibodies in either serum or CSF and a confirmatory plaque reduction neutralization test for WNV **MEETS COVERAGE CRITERIA.**

16) For individuals suspected of having WNV (see Note 10), the use of NAAT for WNV or IFA for WNV-specific IgG antibodies in either serum or CSF **DOES NOT MEET COVERAGE CRITERIA**

17) For individuals suspected of having yellow fever virus (YFV) (see Note 11), the use of NAAT for YFV or serologic assays to detect virus-specific IgM and IgG antibodies, as well as a confirmatory plaque reduction neutralization test for YFV, **MEETS COVERAGE CRITERIA**

18) For the detection of Zika virus, the use of NAAT **MEETS COVERAGE CRITERIA** in the following individuals:

    a) Up to 12 weeks after the onset of symptom for symptomatic (see Note 5) pregnant individuals who have either recently traveled to areas with a risk of Zika (see Note 12) or who have had sex with someone who either lives in or has recently traveled to areas with a risk of Zika (see Note 12).

    b) For infants born from individuals who, during pregnancy, tested positive for Zika virus.

    c) For infants born with signs and symptoms of congenital Zika syndrome (see Note 13) and who have a birthing parent who, during pregnancy, traveled to an area with a risk of Zika (see Note 12).

19) For pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection (see Note 13), Zika virus NAAT (maternal serum and maternal urine) and Zika virus IgM testing (maternal serum), as well as a confirmatory plaque reduction neutralization test for Zika, **MEETS COVERAGE CRITERIA.**

20) For non-pregnant individuals symptomatic for Zika virus infection (see Note 5), NAAT and/or IgM testing for Zika detection **DOES NOT MEET COVERAGE CRITERIA**.

21) For asymptomatic individuals, testing for babesiosis, chikungunya virus, CTF, DENV, ehrlichiosis and/or anaplasmosis, malaria, rickettsial disease, TBRF, WNV, YFV, or Zika virus during a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: Typical signs and symptoms of babesiosis can include hemolytic anemia, splenomegaly, hepatomegaly, jaundice, and nonspecific flu-like symptoms such as fever, chills, body aches, weakness, and fatigue (CDC, 2019a).

Note 2: Typical signs and symptoms of chikungunya include high fever (>102◦F or 39◦C), joint pains (usually multiple joints, bilateral, and symmetric), headache, myalgia, arthritis, conjunctivitis, nausea, vomiting, and maculopapular rash (Staples et al., 2020).

Note 3: Typical signs and symptoms of CTF can include fever, chills, headache, myalgia, malaise, sore throat, vomiting, abdominal pain, and maculopapular or petechial rash (CDC, 2023a).

Note 4: Typical signs and symptoms of dengue can include fever, headache, retro-orbital eye pain, myalgia, arthralgia, erythematous maculopapular rash, petechiae, leukopenia, and nausea and/or vomiting (CDC, 2019b).

Note 5: Typical signs and symptoms of Zika virus infection can include fever, rash, headache, joint pain, conjunctivitis (red eyes), and muscle pain (CDC, 2019d).

Note 6: Typical signs and symptoms of ehrlichiosis and/or anaplasmosis usually begin 5-14 days after an infected tick bite, and they include fever, headache, malaise, myalgia, and shaking chills. Ehrlichiosis can also present with gastrointestinal issues, including nausea, vomiting, and diarrhea (Biggs et al., 2016).

Note 7: Typical signs and symptoms of malaria can include fever, influenza-like symptoms (e.g., chills, headache, body aches), anemia, jaundice, seizures, mental confusion, kidney failure, and acute respiratory distress syndrome (Arguin & Tan, 2019).

Note 8: Typical signs and symptoms of rickettsial diseases (including Rocky Mountain spotted fever, *Rickettsia parkeri* rickettsiosis, *Rickettsia* species 364D rickettsiosis, *Rickettsia* spp (mild spotted fever), and *R. akari* (rickettsialpox)) usually begin 3 – 12 days after initial bite and can include fever, headache, chills, malaise, myalgia, nausea, vomiting, abdominal pain, photophobia, anorexia, and skin rash. *Rickettsia* species 364d rickettsiosis can also present with an ulcerative lesion with regional lymphadenopathy (Biggs et al., 2016).

Note 9: Typical signs and symptoms of tick-borne relapsing fever (caused by *Borrelia hermsii*, *B. mazzottii*, *B. miyamotoi, B. parkeri*, or *B. turicatae*) include recurring febrile episodes that last approximately 3 days separated by approximately 7 days. Nonspecific symptoms that occur in at least 50% of cases include headache, myalgia, chills, nausea, arthralgia, and vomiting (CDC, 2022e).

Note 10: Typical signs and symptoms of WNV include headache, myalgia, arthralgia, gastrointestinal symptoms, and maculopapular rash. Less than 1% of infected individuals develop neuroinvasive WNV with symptoms of meningitis, encephalitis, or acute flaccid paralysis (Nasci et al., 2013).

Note 11: Typical signs and symptoms of yellow fever include symptoms of the toxic form of the disease (jaundice, hemorrhagic symptoms, and multisystem organ failure), as well as nonspecific influenza symptoms (fever, chills, headache, backache, myalgia, prostration, nausea, and vomiting in initial illness) (Gershman & Staples, 2021).

Note 12: The CDC provides information on the risk of Zika in areas in the United States (https://www.cdc.gov/zika/geo/index.html) and outside of the United States and its territories (https://wwwnc.cdc.gov/travel/page/zika-information).

Note 13: Typical signs and symptoms of congenital Zika syndrome can include microcephaly, problems with brain development, feeding problems (e.g., difficulty swallowing), hearing loss, seizures, vision problems, decreased joint movement (i.e., contractures), and stiff muscles (making it difficult to move) (CDC, 2022b).

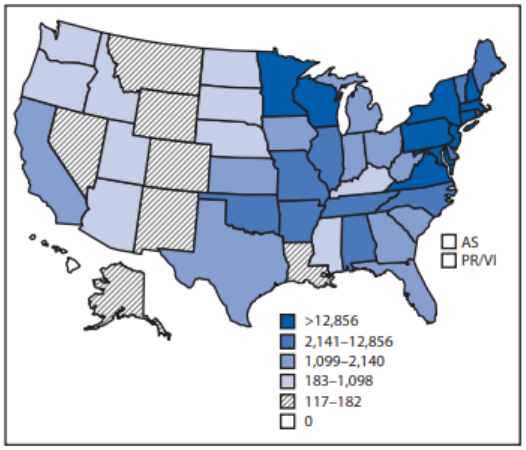
III. Scientific Background

Hematophagous arthropods, such as mosquitoes, ticks, fleas, and mites, can spread opportunistic bacteria, protozoa, and viruses to host organisms when feeding. Numerous outbreaks of arthropod-borne disease have been documented, including plague, an acute febrile disease caused by *Yersinia pestis* through the bite of infected fleas, which resulted in more than 50 million deaths in Europe alone during the "Black Death" outbreak. More than 3000 cases of plague were reported to the World Health Organization (WHO) between 2010 and 2015 with 584 deaths. Today, most cases of plague occur in the Democratic Republic of Congo, Madagascar, and Peru (WHO, 2017).

The Centers for Disease Control and Prevention (CDC) reported a large increase in the number of vector-borne diseases within the United States and its territories between 2004-2016. More than 640,000 cases were reported during that time; in fact, infections of tick-borne bacteria and protozoa more than doubled from 2004 to 2016. "In the United States, 16 vectorborne diseases are reportable to state and territorial health departments, which are encouraged to report them to the National Notifiable Disease Surveillance System (NNDSS). Among the diseases on the list that are caused by indigenous pathogens are Lyme disease (*Borrelia burgdorferi*); West Nile, dengue, and Zika virus diseases; plague (*Yersinia pestis*); and spotted fever rickettsioses (e.g., *Rickettsia rickettsii*). Malaria and yellow fever are no longer transmitted in the United States but have the potential to be reintroduced" (Rosenberg et al., 2018). New vector-borne infections are emerging; for example, two unknown, life-threatening RNA viruses spread by ticks have been identified in the U.S. since 2004. Although both tick- and mosquito-borne diseases are increasing across the U.S., the CDC reports that these two vectors are showing different trends. The mosquito-borne diseases are characterized by epidemics; for example, West Nile Virus is essentially limited to the continental U.S. but has spread rapidly since its introduction to New York in 1999, whereas chikungunya and dengue primarily occur within the U.S. territories. On the other hand, the tick-borne disease increase occurs in the continental U.S. and has experienced a gradual, steady rate increase with Lyme disease comprising 82% of all tick-borne diseases (Rosenberg et al., 2018). Figure 1 and 2 below, taken from Rosenberg et al. (2018), show the reported cases of tickborne and mosquito-borne disease in the United States from 2004-2016.

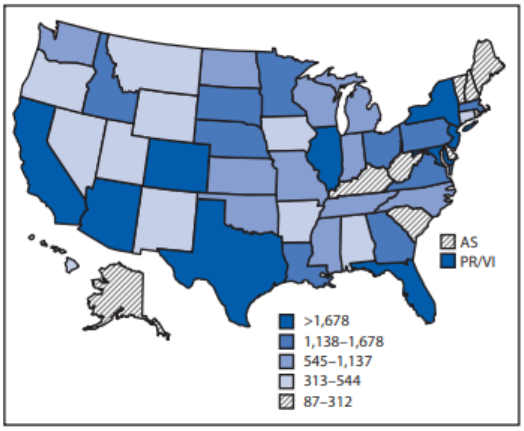
Testing for Vector-Borne Infections, continued

FIGURE 1. Reported cases* of tickborne disease — U.S. states and territories, 2004–2016



Sources: CDC, National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. https://wwwn.cdc.gov/nndss/infectious-tables.html. CDC, Division of Health Informatics and Surveillance. CDC, ArboNET.
Abbreviations: AS = American Samoa; PR/VI = Puerto Rico/U.S. Virgin Islands.
* Data classified by quintile.

FIGURE 2. Reported cases* of mosquitoborne disease — U.S. states and territories, 2004–2016



Sources: CDC, National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. https://wwwn.cdc.gov/nndss/infectious-tables.html. CDC, Division of Health Informatics and Surveillance. CDC, ArboNET.
Abbreviations: AS = American Samoa; PR/VI = Puerto Rico/U.S. Virgin Islands.
* Data classified by quintile.

Rickettsial infections

Rocky Mountain spotted fever (RMSF) is the most common rickettsial infection in the U.S. with 6,248 cases reported to the CDC alone in 2017 (CDC, 2022d). RMSF is caused by *Rickettsia rickettsia*, spread in the U.S. predominantly by *Dermacentor variabilis* (the American dog tick) and *D. andersoni* (the Rocky Mountain wood tick), and can be found throughout North America as well as parts of South America. The Council for State and Territorial Epidemiologists combined RMSF with other rickettsial diseases into the more broad "spotted fever rickettsiosis" designation in 2010 (CDC, 2022d). Besides the obligatory tick bite, typical symptoms of RMSF include fever, headache, and rash with the characteristic rash occurring in approximately 88% to 90% of patients within three to five days of illness. If left untreated, RMSF can be fatal but can easily be treated with antimicrobial therapy upon timely diagnosis. Definitive diagnosis of RMSF cannot usually be made via culture because *Rickettsia* cannot be grown in cell-free culture media since they are obligate intracellular bacteria requiring living host cells. RMSF diagnosis can be made via either skin biopsy prior to treatment with antibiotics or through serologic testing using indirect immunofluorescence assays (IFAs). Immunoglobulin G (IgG) (Biggs et al.) antibodies are more specific than immunoglobulin M (IgM) antibodies since the latter can give false-positive results due to cross-reactivity with other bacterial pathogens. A drawback of IFA is that usually it is unreliable for the first five days of infection until antibody levels are high enough for detection. The CDC and major clinical labs do offer a polymerase chain reaction (PCR)-based assay for RMSF (Sexton & McClain, 2023a).

Since 2001, thirteen more human rickettsiae belonging to the spotted fever group (SFG) have been identified. All SFGs can cause fever, headache, and myalgia and are arthropod-borne (primarily ticks and mites). Most patients with an SFG display a rash and/or a localized eschar. Rickettsialpox, caused by *R. akari*, is transmitted from the bite of a house mouse mite, usually after mouse extermination programs

result in a decrease of the mite's food supply. Rickettsialpox is typically a relatively mild disease that can resolve itself without treatment within three weeks, but treatment hastens improvement. Rickettsiosis can also be due to infection with *R. parkeri*, *R. amblyommii*, and *Rickettsia* species 364D (also called *R. philipii*). Isolation of SFG rickettsiae is rare in clinical practice due to the difficulty of obtaining culture; consequently, serology, immunologic detection from tissue, and PCR are more often used for diagnosis. Microimmunofluorescent (MIF) antibody tests, enzyme-linked immunosorbent assays (ELISAs), and Western blot immunoassays can be used to detect convalescent IgG and IgM antibodies, but these methods can only be used at least 10-14 days after the onset of illness when antibody concentrations are high enough for detection. PCR is a very specific technique. PCR using tissue samples has higher specificity than whole blood PCR. Immunologic detection from a tissue biopsy requires the use of special laboratory equipment so it is not as frequently used as either the serologic or PCR detection methods (Sexton & McClain, 2023c).

Ehrlichiosis and Anaplasmosis

Human ehrlichiosis was first reported in 1986, and the causative agent for human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, was identified in 1994. Both ehrlichiosis and anaplasmosis are transmitted from the bite of infected ticks and have similar clinical and laboratory manifestations. Ehrlichiosis can be caused by *Ehrlichia chaffeensis, E. ewingii, and E. muris.* Typically, patients have a fever within an incubation period of one to two weeks. Other symptoms can include malaise, myalgia, headache, chills, gastrointestinal distress, and cough. Both leukopenia and thrombocytopenia can occur. Diagnosis via culture is extremely difficult. "Until 1995, only two isolates of *E. chaffeensis* had been recovered from humans; in both cases, this process required over 30 days of cultivation. The isolation of *A. phagocytophilum* from three additional patients has been accomplished using a cell culture system derived from human promyelocytic leukemia cells (Sexton & McClain, 2023b). IFA testing for bacteria-specific antibodies is the most common method for diagnosing ehrlichiosis and anaplasmosis, but similar to rickettsiae, ELISA, PCR, and immunochemical tissue staining can be used as well. Unlike rickettsiosis, ehrlichiosis and anaplasmosis can also be detected by the presence of characteristic intraleukocytic morulae in a peripheral blood smear or buffy coat smear (Sexton & McClain, 2022).

Borrelia Infections

Besides Lyme disease, caused by *Borrelia burgdorferi*, *Borrelia* can cause relapsing fever. Tick-borne relapsing fever (TBRF) in North America is primarily caused by *B. hermsii*, *B. turicatae*, *B. parkeri, B. miyamotoi,* and *B.* mazzottii, and louse-borne relapsing fever (LBRF) is an infection caused by *B. recurrentis (Barbour, 2020; Miller et al., 2018)*. The characteristic feature of these infections is the relapsing fever due to cyclical spirochetemia caused by antigenic variation of the spirochetes. Each bout of fever lasts 3 to 12 days with temperatures ranged from 39°C to 43°C (102.2°F to 109.4°F). Visual analysis by Giemsa or Wright staining blood smears taken during a febrile episode is common practice. PCR can also be used on a variety of samples, including cerebrospinal fluid (CSF), blood, tissue, or even culture medium. According to the CDC, "a change in serology results from negative to positive, or the development of an IgG response in the convalescent sample, is supportive of a TBRF diagnosis" (CDC, 2022c). One exception is using antibodies to the GlpQ protein characteristic of these *Borrelia* species but not to *B. burgdorferi* (Lyme disease) (Barbour, 2020).

Protozoa infections

Babesiosis is due to primarily *Babesia microti* in the U.S, but *B. divergens* and *B. venatorum* are the primary causative agents of babesiosis in Europe and China, respectively. The incubation period of *Babesia* depends on the mode of transfection: 1-4 weeks following a tick bite; the incubation period after transfusion of contaminated blood products usually or three to seven weeks but ranges from one week to six months. The most common symptoms of infection include a fever, fatigue, malaise, chills, sweats, headache, and myalgia. Immunocompromised individuals can develop relapsing babesiosis due to an absent or impaired production of antibodies with approximately 20% mortality rate for patients who develop relapsing babesiosis. Most patients with babesiosis are also co-infected with other tick-borne bacterial pathogens. "Preferred tools for diagnosis of babesiosis include blood smear for identification of Babesia organisms and polymerase chain reaction (PCR) for detection of Babesia DNA. Serology can be a useful adjunct to blood smear and PCR" (Krause & Vannier, 2023). Serology is not ideal in diagnosing an acute infection since antibody concentrations remain elevated post-recovery.

Plasmodium falciparum, P. vivax, and *P. ovale* are responsible for malaria. They are spread by the bite of an *Anopheles* mosquito where their sporozoites infect the liver within one to two hours. Within the hepatocyte, they form merozoites. Upon rupturing into the bloodstream, the merozoites infect red blood cells for trophozoite formation, causing the erythrocytic stage of the life cycle where additional merozoites are released. During this stage of the cycle, the symptoms of malaria, including fever, occur. This process usually takes 12 to 35 days, but clinical manifestations can be delayed in individuals with partial immunity or those who are taking ineffective prophylaxis. Other initial symptoms can include irregular heartbeat, cough, anorexia, gastrointestinal distress, sweating, chills, malaise, arthralgia, and myalgia. Malaria, if left untreated, can also include acidosis, hypoglycemia, severe anemia, renal and hepatic impairment, edema, and death (Cohee & Seydel, 2023). Parasite-based diagnosis may include microscopic examination of blood smears, which can often identify the species of *Plasmodium* as well as the parasite density, and antigen-based tests. Rapid diagnostic testing (RDT) of the antigens using immunochromatographic methods is available, but the accuracy of the RDT can vary considerably. NAATs can also be used to identify a malarial infection, and NAATs "are typically used as a gold standard in efficacy studies for antimalarial drugs, vaccines, and evaluation of other diagnostic agents" with a "theoretical limit of detection for PCR…estimated at 0.02 to 1 parasite/microL" (Hopkins, 2023). The Mayo Clinic Laboratories indicates that "PCR is an alternative method of malaria diagnosis that allows for sensitive and specific detection of Plasmodium species DNA from peripheral blood. PCR may be more sensitive than conventional microscopy in very low parasitemias, and is more specific for species identification…Malaria PCR can be used in conjunction with traditional blood film or Babesia PCR when the clinical or morphologic differential includes both babesiosis and malaria" "Test ID: LCMAL Malaria, Molecular Detection, PCR, Varies" (2023).

Viral infections

Examples of arthropod-borne viruses (arboviruses) include West Nile virus (WNV), dengue, yellow fever virus (YFV), chikungunya, and Colorado tick fever virus. In the United States, WNV is the most common arbovirus reported to the CDC. In 2016, 96% of the reported 2,240 cases of domestic arboviruses were WNV with 61% of the WNV cases reported being neuroinvasive. Neuroinvasive WNV includes

meningitis, encephalitis, and acute flaccid paralysis (Burakoff et al., 2018). In general, most infected individuals are asymptomatic with only 20-40% of infected patients showing any characteristic symptoms of WNV, including fever, headache, malaise, myalgia, anorexia, and rash. Diagnosis of WNV of a symptomatic individual usually occurs with a WNV IgM antibody capture ELISA (MAC-ELISA) assay. A patient with symptoms of a neurologic infection does require a lumbar puncture. Confirmatory testing can include a plaque reduction neutralization test (PRNT). PCR testing is primarily used with immunocompromised patients who have delayed or absent antibody production, patients with a history of prior flavivirus infections, and blood donors who may be asymptomatic (Lyle R. Petersen, 2023).

Dengue virus (DENV) infection is a result of being bitten by an infected *Aedes aegypti* or *A. albopictus* mosquito. Four distinct DENV types of *Flavivirus* are known: DENV-1, DENV-2, DENV-3, and DENV-4. DENV is endemic throughout much of the tropical regions of the world, but the only region of the U.S. endemic for DENV is Puerto Rico. The last major outbreak occurred in Puerto Rico in 2010 where 26,766 cases of suspected DENV were reported and 47% of all laboratory tested specimen were positive (CDC, 2019b, 2023c). "Dengue fever…is an acute febrile illness defined by the presence of fever and two or more of the following but not meeting the case definition of dengue hemorrhagic fever: headache, retro-orbital or ocular pain, myalgia and/or bone pain, arthralgia, rash, hemorrhagic manifestations…[and] leukopenia. The cardinal feature of dengue hemorrhagic fever is plasma leakage due to increased vascular permeability as evidenced by hemoconcentration (≥20 percent rise in hematocrit above baseline), pleural effusion, or ascites. DHF [dengue hemorrhagic fever] is also characterized by fever, thrombocytopenia, and hemorrhagic manifestations…. (Thomas et al., 2023)." Laboratory diagnostic testing includes direct detection of viral components in serum or indirect serologic assays. "Detection of viral nucleic acid or viral antigen has high specificity but is more labor intensive and costly; serology has lower specificity but is more accessible and less costly" (Thomas et al., 2023). Culture testing as a diagnostic tool usually is time prohibitive.

Zika virus is a mosquito-borne illness discovered in Uganda in 1947 but has since spread across Asia and to the Americas. Zika infection has been tied to several birth defects. The first human cases of Zika were detected in 1952. Prior to 2007, at least 14 cases of Zika had been documented. Symptoms of Zika are similar to those of many other diseases; therefore, many cases may not have been recognized (CDC, 2019c). The most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis (CDC, 2019c). The illness is usually mild with symptoms beginning 2-7 days after being bitten by an infected mosquito, lasting for several days to a week. Most individuals infected with Zika virus are unaware of the infection, as only a maximum of 25% of people infected will exhibit symptoms(CDC, 2019c; LeBeaud, 2021). Diagnosis of the Zika virus is definitively established through reverse-transcription polymerase chain reaction (RT-PCR) for Zika virus RNA in all symptomatic patients. Aside from pregnant individuals who have traveled to an at risk area, asymptomatic patients are typically not tested (LeBeaud, 2021).

Colorado tick fever virus (CTFV) is a *Reoviridae* transmitted primarily by the Rocky Mountain wood tick (*Dermacentor andersoni*) in the western U.S. and Canada. Transmission of CTFV has also been reported in blood transfusions. The incubation period can last up to 14 days, and symptoms include fever, headache, chills, myalgia, leukopenia, and prostration. Only 15% of symptomatic patients demonstrate a

rash. Serologic tests are usually not helpful until at least 10-14 days for antibody production whereas real-time PCR (RT-PCR) can be used on the first day of symptoms (L. R. Petersen, 2023).

Yellow fever, occurring primarily in sub-Saharan Africa and South America, is a flavivirus spread by mosquitoes that causes hemorrhagic fever with a high fatality rate. An outbreak in Brazil in January-March 2018 resulted in 4 of 10 patients infected with YFV dying. None of those showing symptoms had been vaccinated against YFV. Yellow fever causes hemorrhagic diathesis due to decreased synthesis of vitamin K-dependent coagulation factors as well as hepatic dysfunction, renal failure, and coagulopathy. Yellow fever diagnosis is typically made by a serologic test using an ELISA-IgM assay; however, this assay does cross-react with other flaviviruses and with the YFV vaccination. Rapid diagnostic testing using either PCR or immunoassay is available. Viral isolation and culture can be performed, but it requires inoculation of mosquitoes or mammalian cell culture. Tissue biopsy, such as liver, cannot be performed on the living patient due to possible fatal hemorrhaging; biopsy would be performed during the post-mortem workup (Wilder-Smith, 2023).

Chikungunya virus, endemic in many tropical and subtropical regions of the world, is transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Within the U.S., chikungunya is prevalent in Puerto Rico where approximately 25% of blood donors were seropositive; it has also been reported in Florida. Both dengue and Zika are transmitted by the same vectors, so these viruses often co-circulate geographically. Chikungunya can cause acute febrile polyarthralgia and arthritis. The predominant testing method for diagnosis of chikungunya is the detection of viral RNA via either RT-PCR or virus serology using either ELISA or IFA. Viral culture is typically not used as a diagnostic tool but is used for epidemiologic research (Wilson & Lenschow, 2023).

Types of Testing

Test	Description	Rationale
Culture	Culture growth depends on the pathogen being studied. If the pathogen is an obligate intracellular organism, then it must be isolated using more sophisticated cell culture techniques. In many circumstances, culture is used for research and/or epidemiology rather than as a diagnostic tool (Biggs et al., 2016; Miller et al., 2018).	At times, culture testing is not as sensitive as either NAAT or serologic testing and can be time-intensive when treatment should not be delayed. Depending on the organism, this may require high biosafety level laboratory for culture growth (Biggs et al., 2016).
Indirect immunofluor-escene antibody (IFA) assays	IFA is a serologic assay that can be used to test for the presence of antibodies, such as IgG and IgM, reactive against the pathogen (Biggs et al., 2016).	Depending on the pathogen, IFA can be a useful tool. At times, though, it can cross-react with either a prior vaccination or infection (Wilder-Smith, 2023). An acute infection can often be determined by performing IFA in both the acute phase and convalescent phase where at least a fourfold increase in antibodies is indicative of an acute infection (Biggs et al., 2016).
Darkfield microscopy	Darkfield microscopy can be used to detect the presence of microorganisms, such as motile spirochetes (Miller et al., 2018).	This technique is not widely available, and transport of sample must be done

Test	Description	Rationale
		immediately if testing of motile specimen is desired (Miller et al., 2018).
Blood-smear microscopy	Blood-smear microscopy can be either thick or thin and is typically performed on a sample stained with an eosin-azure-type dye, such as Giemsa, to look at intracellular structures or morphological features (Biggs et al., 2016).	This technique should be performed by an experienced microscopist since it can be inconsistent. As compared to other techniques, this technique is relatively inexpensive (Biggs et al., 2016).
Nucleic acid amplification testing (NAAT)	NAATs can include polymerase chain reaction (PCR), real-time PCR (RT-PCR), or other enzyme-dependent amplification testing for the presence of nucleic acids (DNA or RNA).	NAATs can be specific and sensitive; however, they may not be available at all laboratories and/or can be costly. Some NAATs are available as rapid diagnostic tools. NAATs have been used on serum, whole blood, tissue, CSF, and even formalin-fixed, paraffin-embedded biopsies from autopsy tissues. The sensitivity of the technique can vary depending on the sample; for example, whole blood PCR for *R. rickettsii* is less sensitive than a similar sample test for *E. chaffeensis* (Biggs et al., 2016).

Analytical Validity

The use of antibodies to detect and diagnose arthropod-associated infections and diseases is a common practice. Johnson et al. (2000) first reported the use of monoclonal antibody-based capture ELISA testing for a variety of alphaviruses, including chikungunya, flaviviruses, including dengue and yellow fever, and bunyaviruses. The researchers concluded, "IgG ELISA results correlated with those of the standard plaque-reduction neutralization assays. As expected, some test cross-reactivity was encountered within the individual genera, and tests were interpreted within the context of these reactions. The tests were standardized for laboratory diagnosis of arboviral infections, with the intent that they be used in tandem with the corresponding IgM antibody-capture ELISAs" (Johnson et al., 2000). Kalish and associates also demonstrated that IgG and/or IgM antibody responses can still occur up to 20 years post-infection; consequently, a rise in antibody titer does not necessarily indicate a current, acute infection (Kalish et al., 2001).

Granger and Theel (2019) published an evaluation of two enzyme-linked immunosorbent assays and a rapid immunochromatographic assay for the detection of IgM antibodies to Zika virus. This article states that five serological assays have been approved by the FDA in an emergency use situation and include the Chembio DPP Zika IgM system (a rapid immunochromatographic assay), the InBios ZIKV Detect 2.0 IgM antibody capture enzyme-linked immunosorbent assay, and the InBios ZIKV Detect MAC-ELISA. These three serologic assays were evaluated, using 72 samples, based on the identification of neutralizing antibodies to Zika virus, dengue virus, or West Nile virus. "The Chembio DPP Zika ICA and InBios ZIKV 2.0 MAC-ELISA showed 95% specificity in 22 ZIKV/DENV-seronegative specimens and in 13 samples positive for NAbs to non-ZIKV flaviviruses. Comparatively, the InBios ZIKV MAC-ELISA was "presumptive" or "possible Zika positive" in 8 of 12 WNV or DENV PRNT-positive samples and in 12 of 22 PRNT-seronegative sera (Granger & Theel, 2019)." The authors conclude that by replacing the InBios

ZIKV MAC-ELISA with the InBios ZIKV 2.0 MAC-ELISA, testing burden will be minimized on laboratories performing PRNT for the identification of neutralizing antibodies.

Leski et al. (2020) performed a 2020 study published in Malaria Journal that compared traditional diagnostic methods such as rapid diagnostic tests (RDTs) and DNA-based methods to polymerase chain reaction (PCR). The results indicated consistency with "previous observations that PCR-based tests have a significantly higher sensitivity when compared with both microscopy and RDTs" Leski et al. (2020).

Mathison and Pritt (2017) reviewed current standards for malaria testing and the most used methods for laboratory diagnosis. The most common tests "are microscopic examination of stained blood flims and detection of parasite antigen or nucleic acid… Rapid antigen detection methods and molecular amplification tests are also increasingly employed for malaria diagnosis and are useful adjunctive tests." According to the algorithm developed in "Update on Malaria Diagnostics and Test Utilization," NAAT tests are one of three tests recommended for use if malaria is suspected based on clinical findings and exposure history (Mathison & Pritt, 2017).

Kim et al. (2018) had also developed a rapid diagnostic test (RDT) for detecting IgG/IgM antibodies against Zika virus using "monoclonal antibodies to the envelope (E) and non-structural protein (NS1)." The diagnostic accuracy of this kit was "fairly high; sensitivity and specificity for IgG was 99.0 and 99.3%, respectively, while for IgM it was 96.7 and 98.7%, respectively." However, there were cross reactions with the dengue virus evaluated using anti-Dengue Mixed Titer Performance Panel (PVD201), "in which the Zika RDT showed cross-reactions with [dengue virus] in 16.7% and 5.6% in IgG and IgM, respectively." This research could potentially enable the rapid diagnostic test to be preferable to the traditional RT-PCR in endemic areas (Kim et al., 2018).

Clinical Utility and Validity

In 2013, Kato and colleagues tested the sensitivity of two different RT-PCR-based assays for Rickettsia—PanR8, an assay that tests for Rickettsia in general, and RRi6, an assay specific for *R. rickettsii*. Both of these methods were more sensitive in testing for Rickettsia than the nested PCR method of the CDC; moreover, both of these methods are faster than the nested PCR method (1 hr versus 1-2 days, respectively) (Kato et al., 2013). These results were corroborated in 2014 by Denison and colleagues. They used a multiplex PCR assay to correctly identify all cell controls for *R. rickettsii*, *R. parkeri*, and *R. akari*; moreover, no false-positive results were reported using this methodology. "This multiplex real-time PCR demonstrates greater sensitivity than nested PCR assays in FFPE [formalin-fixed, paraffin-embedded] tissues and provides an effective method to specifically identify cases of Rocky Mountain spotted fever, rickettsialpox, and *R. parkeri* rickettsiosis by using skin biopsy specimens" (Denison et al., 2014).

The FDA has approved the use of the BinaxNOW malaria test for screening and diagnosing malaria. Even though, this testing method is considerably faster than other methods (as low as 1.1-1.7 hours complete turnaround time (Ota-Sullivan & Blecker-Shelly, 2013)), the use of BinaxNOW in non-endemic areas is a point of controversy due to relatively low sensitivity (84.2%) and for misclassifying Plasmodium falciparum malaria as non-falciparum (Dimaio et al., 2012). Moreover, it has been reported that Salmonella typhi can give a false-positive for malaria using the BinaxNOW test (Meatherall et al., 2014).

van Bergen et al. (2021) evaluated a novel real-time PCR assay for clinical validity. The authors used reference samples, patient samples, and synthetic controls. The analytical performance details of the MC004 assay were considered: "analytical specificity, limit of detection, the ability to detect mixed infections, and the potential to determine the level of parasitaemia of P. falciparum, including assessment of within-run and between-run precisions." The authors reported "zero false positive or false negative results." As far as precision, "the within-run and between-run precisions were less than 20% CV at the tested parasitaemia levels of 0.09%, 0.16%, 2.15% and 27.27%." Based on these results, the authors reported that "the entry of PCR-based techniques into malaria diagnostics has improved the sensitivity and specificity of the detection of Plasmodium infections… Based upon the analytical performance characteristics that were determined, the MC004 assay showed performance suitable for use in clinical settings, as well as epidemiological studies" (van Bergen et al., 2021).

Akoolo et al. (2017) compared qPCR results in the detection of Babesia infection against currently available non-NAAT tests (FISH and microscopy). Blood samples were analyzed from 192 patients. The researchers report that "Of 28 samples that were positive by FISH, 27 (96%) were also positive by qPCR indicating high congruency between nucleic acid-based tests. Interestingly, of 78 asymptomatic samples not tested by FISH, 22 were positive by our qPCR" (Akoolo et al., 2017). Overall, the qPCR method was found to have a sensitivity of 96.2% and a specificity of 70.5%. The authors conclude, "Robust qPCR using specific probes can be highly useful for efficient and appropriate diagnosis of babesiosis in patients in conjunction with conventional diagnostics, or as a stand-alone test, especially for donated blood screening" (Akoolo et al., 2017).

Reynolds et al. (2017) examined the 2016 United States Pregnancy Registry to estimate the proportion of birth defects of pregnant women exposed to Zika, and out of 972 pregnancies with laboratory evidence of a possible Zika infection, 51 had birth defects (5%). Of the 250 confirmed infections, 24 had birth defects. Similarly, Shiu et al. (2018) evaluated the screening results of the Zika virus in Miami-Dade County in Florida. Of 2327 women screened for Zika, 86 had laboratory evidence of infection, and 2 had congenital Zika "syndrome" (Zika-caused birth defects) (Shiu et al., 2018).

IV. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

(continued on next page)

Diagnosis and Management of Tickborne Rickettsial Diseases (Biggs et al., 2016): In 2016, the CDC released their guidelines and recommendations concerning Rickettsial diseases, including Rocky Mountain spotted fever, in the MMWR. The table below summarizes their recommended diagnostic tests for tickborne rickettsial diseases:

TABLE 4. Recommended diagnostic tests for tickborne rickettsial diseases

Disease	PCR			Microscopy for morulae detection	IFA assay for IgG antibodies (acute and convalescent)*
	Whole blood	Eschar biopsy or swab	Rash biopsy		
Rocky Mountain spotted fever	Yes†	—	Yes	—	Yes
Rickettsia parkeri rickettsiosis	—	Yes	Yes	—	Yes
Rickettsia species 364D rickettsiosis	—	Yes	—	—	Yes
Ehrlichia chaffeensis ehrlichiosis (human monocytic ehrlichiosis)	Yes	—	—	Yes	Yes
Ehrlichia ewingii ehrlichiosis	Yes	—	—	Yes	Yes
Ehrlichia muris-like agent ehrlichiosis	Yes	—	—	—	Yes
Human anaplasmosis (human granulocytic anaplasmosis)	Yes	—	—	Yes	Yes

Abbreviations: IFA = indirect immunofluorescence antibody; IgG = immunoglobulin G; PCR = polymerase chain reaction.
* IFA assay is insensitive during the first week of illness for most tickborne rickettsial diseases; a sample should be collected during this interval (acute specimen), and a second sample should be collected 2–4 weeks later (convalescent specimen) for comparison. Elevated titers alone are not sufficient to diagnose infection with tickborne rickettsial diseases; serial titers are needed for confirmation. Demonstration of at least a fourfold rise in antibody titer is considered confirmatory evidence of acute infection.
† PCR of whole blood samples for *Rickettsia rickettsii* has low sensitivity; sensitivity increases in patients with severe disease.

To summarize their recommendations, even though indirect immunofluorescence antibody assays (IFAs) are insensitive typically during the first week of an acute infection, they are the standard reference for tickborne rickettsial infections and that a minimum of two tests are to be performed for diagnosis. Usually, one sample is taken early after the initial symptoms are present, and a second sample is taken 2-4 weeks later. A minimum of a fourfold rise in antibody titer is required to confirm diagnosis. In cases of ehrlichiosis and anaplasmosis, during the first week, PCR amplification can be used on whole blood for diagnosis, but PCR has low sensitivity in Rocky Mountain spotted fever except in patients with severe disease. Morulae detection via either blood-smear or buffy-coat preparation microscopy can also be indicative of ehrlichiosis or anaplasmosis. However, "Rickettsiae cannot be isolated with standard blood culture techniques because they are obligate intracellular pathogens; specialized cell culture methods are required. Because of limitations in availability and facilities, culture is not often used as a routine confirmatory diagnostic method for tickborne rickettsial diseases" (Biggs et al., 2016).

Tick-borne relapsing fever (TBRF) (CDC, 2018c): In the U.S., TBRF can be caused by *Borrelia hermsii*, *B. parkerii,* and *B. turicatae* with *B. hermsii* being the most common causative agent. TBRF often presents with a relapsing nature (usually ~3 days per febrile episode followed by an afebrile period of approximately one week). Moreover, "Spirochetemia (spirochetes in blood) in TBRF patients often reaches high concentrations (>10^6 spirochetes/ml). Thus, microscopy is a useful diagnostic tool for TBRF. The diagnosis of TBRF may be based on direct microscopic observation of relapsing fever spirochetes using dark field microscopy or stained peripheral blood smears. Spirochetes are more readily detected by microscopy in symptomatic, untreated patients early in the course of infection. Other bacteria, such as Helicobacter, may appear morphologically similar, so it is important to consider clinical and geographical characteristics of the case when making a diagnosis of TBRF based on microscopy. Additional testing, such as serology or culture, is recommended."

CDC acknowledges that "Serologic testing for TBRF is not standardized and results may vary by laboratory. Serum taken early in infection may be negative, so it is important to also obtain a serum sample during the convalescent period (at least 21 days after symptom onset). A change in serology results from negative to positive, or the development of an IgG response in the convalescent sample, is supportive of a TBRF diagnosis. However, early antibiotic treatment may limit the antibody response. Patients with TBRF may have false-positive tests for Lyme disease because of the similarity of proteins between the causative organisms. A diagnosis of TBRF should be considered for patients with positive Lyme disease serology who have not been in areas endemic for Lyme disease. Incidental laboratory findings include normal to increased white blood cell count with a left shift towards immature cells, a mildly increased serum bilirubin level, mild to moderate thrombocytopenia, elevated erythrocyte sedimentation rate (ESR), and slightly prolonged prothrombin time (Reynolds et al.) and partial thromboplastin time (PTT)" (CDC, 2022e).

Colorado Tick Fever (CTF) (CDC, 2023b): As of 2015, CTF was reportable in Arizona, Colorado, Montana, Oregon, Utah, and Wyoming. "Laboratory diagnosis of CTF is generally accomplished by testing of serum to detect viral RNA or virus-specific immunoglobulin (Ig) M and neutralizing antibodies. Antibody production can be delayed with CTF, so tests that measure antibodies may not be positive for 14–21 days after the onset of symptoms. RT-PCR (reverse-transcriptase polymerase chain reaction) is a more sensitive test early in the course of disease. CTF testing is available at some commercial and state health department laboratories and at CDC. Contact your state or local health department for assistance with diagnostic testing. They can help you determine if samples should be sent to the CDC Arbovirus Diagnostic Laboratory for further testing (CDC, 2023b)."

Babesiosis (CDC, 2019a): According to the CDC website, the most recent update about babesiosis for health professionals is from 2012 (with revision in 2018). Diagnosis can be challenging due to the nonspecific clinical manifestations of the disease. "For acutely ill patients, the findings on routine laboratory testing frequently include hemolytic anemia and thrombocytopenia. Additional findings may include proteinuria, hemoglobinuria, and elevated levels of liver enzymes, blood urea nitrogen, and creatinine. If the diagnosis of babesiosis is being considered, manual (non-automated) review of blood smears should be requested explicitly. In symptomatic patients with acute infection, *Babesia* parasites typically can be detected by light-microscopic examination of blood smears, although multiple smears may need to be examined. Sometimes it can be difficult to distinguish between *Babesia* and *Plasmodium* (especially *P. falciparum*) parasites and even between parasites and artifacts (such as stain or platelet debris). Consider having a reference laboratory confirm the diagnosis—by blood-smear examination and, if indicated, by other means, such as molecular and/or serologic methods tailored to the setting/species (CDC, 2019)."

Malaria (Arguin & Tan, 2017, 2019): The CDC considers smear microscopy as the gold standard in diagnosing malaria since it can determine the species, identify the stage of parasitic life-cycle, and quantify the parasitemia. The CDC states, "Blood smear microscopy remains the most important method for malaria diagnosis. Microscopy can provide immediate information about the presence of parasites, allow quantification of the density of the infection, and allow determination of the species of the malaria parasite—all of which are necessary for providing the most appropriate treatment. Microscopy

results should ideally be available within a few hours. These tests should be performed immediately when ordered by a health care provider. They should not be saved for the most qualified staff to perform or batched for convenience. In addition, these tests should not be sent out to reference laboratories with results available only days to weeks later. Assistance with speciation of malaria on smears is available from CDC" (Arguin & Tan, 2019). The CDC also notes that rapid diagnostic tests (RDTs) for malaria can detect malaria parasitic antigens. However, "RDTs offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not immediately available. Although RDTs can detect malaria antigens within minutes, they have several limitations. RDTs cannot distinguish between all of the *Plasmodium* species that affect humans, they may be less sensitive than expert microscopy or PCR for diagnosis, they cannot quantify parasitemia, and an RDT-positive test result may persist for days or weeks after an infection has been treated and cleared. Thus, RDTs are not useful for assessing response to therapy. Both positive and negative RDT results must always be confirmed by microscopy. Microscopy confirmation of the RDT result should occur as soon as possible because the information on the presence, density, and parasite species is critical for optimal management of malaria"(Arguin & Tan, 2019). Regarding PCR, the CDC states that "Although these tests are more sensitive than routine microscopy, results are not usually available as quickly as microscopy results, thus limiting the utility of this test for acute diagnosis and initial clinical management. Use of PCR testing is encouraged to confirm the species of malaria parasite and detect mixed infections" (Arguin & Tan, 2019).

The CDC also provided an update to malaria diagnosis in 2018. Although microscopy remained the "gold standard" for confirmation of malaria, other tests such as RDTs and PCR-based tests remained useful in certain situations (namely if microscopy is unavailable). PCR is considered most useful for "confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT." Finally, the CDC recommends that all cases of malaria be evaluated for drug resistance, typically through molecular characterization (PCR, gene sequencing) or in vitro tests (CDC, 2018b).

Chikungunya (Staples et al., 2017, 2020): In the CDC Yellow Book, concerning the chikungunya virus, they recommend that "the differential diagnosis of chikungunya virus infection depends on the clinical signs and symptoms as well as where the person was suspected of being infected." The other diseases to consider include: Zika, malaria, leptospirosis, parvovirus, group A *Streptococcus,* rubella, measles, dengue, enterovirus, adenovirus, alphavirus infections, post-infectious arthritis, and rheumatic conditions. Laboratory diagnosis is done by serum testing for detection of virus, viral nucleic acids, or virus-specific IgM and neutralizing antibodies. "During the first week after onset of symptoms, chikungunya can often be diagnosed by performing viral culture or nucleic acid amplification on serum. Virus-specific IgM and neutralizing antibodies normally develop toward the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent-phase samples should be obtained from patients whose acute-phase samples test negative. Testing for chikungunya virus is performed at CDC, several state health department laboratories, and several commercial laboratories (Staples et al., 2017, 2020)."

West Nile Virus (WNV) (Nasci et al., 2013): "WNV infections are most frequently confirmed by detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or cerebrospinal fluid (CSF). The presence of

anti-WNV IgM is usually good evidence of recent WNV infection, but may indicate infection with another closely related flavivirus (e.g., St. Louis encephalitis). Because anti-WNV IgM can persist in some patients for >1 year, a positive test result occasionally may reflect past infection unrelated to current disease manifestations. Serum collected within 8 days of illness onset may lack detectable IgM, and the test should be repeated on a convalescent-phase sample. IgG antibody generally is detectable shortly after the appearance of IgM and persists for years. Plaque-reduction neutralization tests (PRNT) can be performed to measure specific virus-neutralizing antibodies. A fourfold or greater rise in neutralizing antibody titer between acute- and convalescent-phase serum specimens collected 2 to 3 weeks apart may be used to confirm recent WNV infection and to discriminate between cross-reacting antibodies from closely related flaviviruses." NAAT may not be suitable in most cases since the concentrations of WNV RNA are so low by the time a patient begins to show symptoms of infection; however, NAAT may be suitable in immunocompromised individuals who have either delayed or absent antibody development.

Yellow Fever Virus (YFV) (Gershman & Staples, 2021): Isolation of the virus or NAAT should be performed as early as possible in suspected cases of YFV. "By the time more overt symptoms are recognized, the virus or viral RNA may no longer be undetectable. Therefore, virus isolation and nucleic acid amplification should not be used to rule out a diagnosis of YF… Serologic assays to detect virus-specific IgM and IgG antibodies (*sic*). Because of cross-reactivity between antibodies raised against other flaviviruses, more specific antibody testing, such as a plaque reduction neutralization test, should be done to confirm the infection (Gershman & Staples, 2021)." Since YFV is a nationally notifiable disease, clinicians should contact their state and/or local health departments according to their respective local, state, and/or federal guidelines. As of April of 2021, "Sanofi Pasteur announced that YF-VAX (yellow fever vaccine) is once again available for purchase in the United States. Providers with a current Yellow Fever Vaccination Stamp issued by their state or territorial health department may now order YF-VAX from the manufacturer" (Gershman & Staples, 2021).

Dengue (CDC, 2020, 2023c): Diagnosis of dengue can be via isolation of virus, serological tests such as immunoassays, and molecular methods, including RT-PCR. The CDC recommends testing symptomatic individuals that have recently traveled to or lives in areas where dengue is transmitted, as well as symptomatic pregnant women with possible dengue or Zika exposure. The CDC states that dengue virus testing is not recommended for asymptomatic patients and is not recommended for preconception screening. The CDC's testing algorithm for dengue is as follows:

"Patients with symptoms consistent with dengue can be tested with both molecular and serologic diagnostic tests during the first 7 days of illness. After the first 7 days of illness, test only with serologic diagnostic tests" (CDC, 2020).

Zika / Dengue Virus (CDC): The CDC released updated guidelines associated with Zika and Dengue testing for pregnant individuals. For asymptomatic pregnant individuals "living in or with recent travel to the U.S. and its territories, routine Zika virus testing is not currently recommended . . . recent travel to an area with risk of Zika outside the U.S. and its territories, Zika virus testing is not routinely recommended, but NAAT testing may still be considered up to 12 weeks after travel . . . Zika virus serologic testing is not recommended . . . There is notable cross-reactivity between dengue IgM and Zika IgM antibodies in

serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive."

For symptomatic pregnant individuals who had "recent travel to areas with active dengue transmission and a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset. The following diagnostic testing should be performed at the same time: Dengue and Zika virus NAAT testing on a serum specimen, Zika virus NAAT on a urine specimen, and IgM testing for dengue only. Zika virus IgM testing is not recommended for symptomatic pregnant individuals. Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection. There is also notable cross-reactivity between dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive. If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results. If the dengue NAAT is positive, this provides adequate evidence of a dengue infection, and no further testing is indicated. If the IgM antibody test for dengue is positive, this is adequate evidence of a dengue infection, and no further testing is indicated."

For symptomatic pregnant individuals who have had sex with someone who lives in or recently traveled to areas with a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset. Only Zika NAAT should be performed. If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results."

The following should be considered guidance for pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection who live in or traveled to areas with a risk of Zika during her pregnancy. "Zika virus NAAT and IgM testing should be performed on maternal serum and NAAT on maternal urine. If the Zika virus NAATs are negative and the IgM is positive, confirmatory PRNTs should be performed against Zika and dengue. If amniocentesis is being performed as part of clinical care, Zika virus NAAT testing of amniocentesis specimens should also be performed and results interpreted within the context of the limitations of amniotic fluid testing. It is unknown how sensitive or specific RNA NAAT testing of amniotic fluid is for congenital Zika virus infection or what proportion of infants born after infection will have abnormalities. Testing of placental and fetal tissues may also be considered."

Per the CDC, "Symptomatic non-pregnant patients should refer to testing guidance for dengue. Zika testing is NOT currently recommended for this group based on the current epidemiology of these viruses . . . As per previous guidance, asymptomatic non-pregnant patients should NOT be tested for dengue or Zika viruses. Zika virus testing should NOT be performed as part of preconception screening" (CDC, 2022f).

The CDC also notes that: "Laboratory testing for congenital Zika virus infection is recommended for infants born to mothers with laboratory evidence of Zika virus infection during pregnancy, and for infants who

have abnormal clinical findings suggestive of congenital Zika virus syndrome and a maternal epidemiologic link suggesting possible transmission, regardless of maternal Zika virus test results" (CDC, 2018a).

Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)
Laboratory Diagnosis of Tick-borne Infections: The information given below outlines the diagnostic procedures for tick-borne infections and is taken from Table 47 of the 2018 IDSA/ASM guidelines.

Bacteria		
Etiologic Agents	**Diagnostic Procedures**	**Optimum Specimens**
Relapsing fever borreliae *Borrelia hermsii* (western US) *Borrelia parkeri* (western US) ***Borrelia turicatae*** **(southwestern US)** ***Borrelia mazzottii*** **(southern US)**	Primary test: Darkfield microscopy or Wright, Giemsa, or Diff-Quik stains of peripheral thin or/ and thick blood smears. Can be seen in direct wet preparation of blood in some cases.	Blood or bone marrow
	Other testing: NAAT, Culture, Serologic testing	Blood or body fluids for NAAT. Serum for culture or serologic testing.
Borrelia miyamotoi (B. miyamotoi **infection, hard tick-borne relapsing fever)**	Primary test: NAAT	Blood
	Serology: EIA for detection of antibodies to recombinant GlpQ antigen	Serum
Anaplasma phagocytophilum **(human granulocytotropic anaplasmosis)**	Primary test: NAAT Alternate Primary (if NAAT is unavailable): Wright or Giemsa stain of peripheral blood or buffy coat leukocytes during week first week of infection.	Blood
	Serology: Acute and convalescent IFA titers for IgG-class antibodies to A. phagocytophilum antibodies	Serum
	Immunohistochemical staining of Anaplasma antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver, and lung)
Ehrlichia chaffeensis **(human monocytotropic ehrlichiosis)** ***Ehrlichia muris*** ***Ehrlichia ewingii***	Primary test: NAAT (Only definitive diagnostic assay for *E. ewingii*) Wright or Giemsa stain of peripheral blood or buffy coat leukocytes smear during first week of infection Immunohistochemical staining of	Whole blood for NAAT Blood for Wright or Giemsa stain

Bacteria		
	Serology: acute and convalescent IFA titers for Ehrlichia IgG-class antibodies	Serum
	Ehrlichia antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)
***Rickettsia rickettsii* (RMSF)** **Other spotted fever group *Rickettsia* spp (mild spotted fever)** ***R. typhi* (murine typhus)** ***R. akari* (rickettsialpox)** ***R. prowazekii* (epidemic typhus)**	Serology: acute and convalescent IFA for *Rickettsia* sp IgM and IgG antibodies	Serum
	NAAT	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)
	Immunohistochemical staining of spotted fever group rickettsiae antigens (up to first 24 h after antibiotic therapy initiated) in formalin-fixed, paraffin-embedded specimens	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)
Protozoa		
Etiologic Agents	**Diagnostic Procedures**	**Optimum Specimens**
Babesia microti *Babesia* spp	Primary test: Giemsa, Wright, Wright-Giemsa stains of peripheral thin and thick blood smears (Giemsa preferred)	Whole blood (EDTA vacutainer tube is a second choice
	Primary test for acute infection: NAAT	Blood
	Serology: acute and convalescent IFA titers for Babesia IgG-class antibodies **NOTE: Not recommended for acute infection.**	Serum
Virus		
Etiologic Agents	**Diagnostic Procedures**	**Optimum Specimens**
Colorado tick fever virus	Serology: IFA titers or complement fixation	Serum
Powassan/deer tick virus	Primary test: IgM capture EIA (available only through state departments of public health)	Serum for EIA
	NAAT	Blood, CSF, brain (biopsy or autopsy) for NAAT

The IDSA/ASM does note that most PCR-based assays for babesiosis only detect *B. microti* even though there are at least three other species of *Babesia* that can cause the infection. "Real time PCR available from CDC and reference labs… Serology does not distinguish between acute and past infection" (Miller et al., 2018).

Their recommendation for the main diagnostic testing for malaria due to *Plasmodium falciparum, P. ovale, P. vivax, P. malariae, and P. knowlesi* is "Stat microscopic examination of Giemsa-stained thick and thin blood films (repeat testing every 12–24 h for a total of 3 exams before ruling out malaria); rapid antigen detection tests followed by confirmatory blood films within 12–24 h." They make the following special remark: "Antigen tests lack sensitivity with low parasitemia and non-falciparum malaria and do not differentiate all species. PCR from some reference laboratories will detect and differentiate all species. Calculation of percentage parasitemia (using thick or thin blood films) is required for determining patient management and following response to therapy (Miller et al., 2018)." Concerning dengue virus DENV, "Plaque reduction neutralization tests (PRNTs) are considered the reference standard for detection of antibodies to arthropod-borne viruses (arboviruses) and provide improved specificity over commercial serologic assays; however, due to the complexity of testing, PRNT is currently only available at select public health laboratories and the CDC." They note that false-positives for antibodies to DENV may not necessarily indicate DENV infection since it can also be indicative of a prior flavivirus infection, such as West Nile virus or Zika virus. They also state that the "Detection of DENV RNA by NAAT is preferred for acutely ill patients. Recently, detection of the DENV NS1 antigen, which is secreted from infected host cells as early as 1 day after symptom onset and up to 10 days thereafter, has become an acceptable alternative to NAAT for diagnosis of acute DENV infection (Miller et al., 2018)."

For West Nile Virus (WNV), they state: "Laboratory diagnosis of WNV, and most other arboviruses, is typically accomplished by detecting virus-specific IgM- and/or IgG-class antibodies in serum and/or CSF." Possible false-positives can occur if a patient has been vaccinated against yellow fever or if they have had a previous infection of another flavivirus. They do note that WNV RNA detection via NAAT can be performed on either the serum or CSF for immunosuppressed patients.

World Health Organization (WHO)

Interim guidance for laboratory testing of Zika and dengue virus published in July 2022 by WHO includes these updated key considerations, recommendations, and good practices:

- ZIKV and DENV infections need to be differentiated from each other, and from other circulating arboviral and non-arboviral pathogens, using laboratory tests.
- Laboratory tests performed and interpretation of results must be guided by the interval between symptom onset or exposure, and the collection of specimens.
- WHO recommends the use of whole blood, serum, or plasma routine diagnostic testing for arboviruses, and urine for ZIKV NAAT testing.
- Molecular assays are the preferred detection method but the period of RNA detectability following infection is limited.

- Interpretation of serologic test results remains challenging because of cross-reactivity and prolonged detection of virus-specific antibodies; their utility depends on the patient's current and prior flavivirus exposures.
- Testing for antibodies to ZIKV and DENV should thus be done with careful consideration of epidemiologic and clinical context.
- For pregnant women, the diagnosis of ZIKV should always be based on laboratory evidence and testing in these patients should not be limited to a subset of samples, even during outbreaks.
- For pregnant women, accurate diagnosis is of particular importance; prolonged detection of RNA in blood and urine may facilitate. confirmation of ZIKV infection in these patients
- ZIKV IgM testing in pregnant women should be used with caution, since a positive test might reflect infection that occurred prior to pregnancy
- ZIKV testing for asymptomatic pregnant women remains challenging because of unknown optimal timing of specimen collection and risks of false positive and false negative results.
- Only laboratory tests that have undergone independent, comprehensive assessment of quality, safety and performance should be used for diagnosing arboviral infections.
- Any testing for the presence of ZIKV, DENV, and other pathogens in the differential diagnosis should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures (WHO, 2022)

American Society for Microbiology (ASM)

The ASM updated guidelines in 2022 on laboratory testing for Zika virus. They state, "Diagnostic testing may be warranted for patients who live in or have recently travelled to an endemic region and are critically ill, hospitalized or pregnant, or infants born to Zika virus positive mothers" (ASM, 2022). The ASM endorses CDC guidelines on Zika as well.

American Academy of Pediatrics 2018 Redbook

Babesiosis (AAP, 2021a): "Acute, symptomatic cases of babesiosis typically are diagnosed by microscopic identification of Babesia parasites on Giemsa- or Wright-stained blood smears… PCR assay is particularly recommended for use in early infection, when parasites are more difficult to visualize on blood smear. However, PCR assay should be used with caution when monitoring response to therapy, because B. microti can be detected for weeks and months after parasites no longer are visualized on blood smear." They do state that antibody testing can be useful in distinguishing between Babesia and Plasmodium infections whenever blood smear examinations and travel histories are inconclusive or for detecting individuals with very low levels of parasitemia.

Non-Lyme Borrelia Infections (AAP, 2021b): Dark-field microscopy and Wright-, Giemsa-, or acridine orange-stained preparations of blood smears can be used to observe the presence of spirochetes in the initial febrile episode, but their presence is more difficult to determine in future recurrences. Both enzyme immunoassay and Western immunoblot analysis can detect serum antibodies; however, "these antibody tests are not standardized and are affected by antigenic variations among and within Borrelia species and strains." As of publication, PCR and antibody-based testing were still under development and were not widely available.

Ehrlichia, Anaplasma, and Related Infections (AAP, 2021e): PCR testing should be performed within the first week of illness to diagnose anaplasmosis, ehrlichiosis, and other Anaplasmataceae infections because doxycycline treatment rapidly decreases the sensitivity of PCR. Consequently, negative PCR results do not necessarily indicate a lack of infection. Occasionally, Giemsa- or Wright staining of blood smears can be performed to identify the presence of the morulae of Anaplasma in the first week of illness. Culture testing for isolation is not performed. "Immunoglobulin (Ig) M serologic assays are prone to false-positive reactions, and IgM… can remain elevated for lengthy periods of time, reducing its diagnostic utility. Serologic testing may be used to demonstrate a fourfold change in IgG-specific antibody titer by indirect immunofluorescence antibody (IFA) assay between paired acute and convalescent specimens taken 2 to 4 weeks apart."

Rocky Mountain Spotted Fever (RMSF) (AAP, 2021h): "The gold standard for serologic diagnosis of RMSF is the indirect immunofluorescence antibody (IFA) test. A negative serologic test result from the acute phase does not rule out a diagnosis of RMSF in any case… A fourfold or greater increase in antigen-specific IgG between acute and convalescent sera obtained 2 to 4 weeks apart confirms the diagnosis (6 weeks for convalescent serum for Rickettsia africae)."

Rickettsialpox (AAP, 2021g): Rickettsialpox can be mistaken for other rickettsial infections. Ideally, the use of R. akari-specific antigen is recommended for serologic diagnosis, but it has limited availability. Otherwise, indirect IFA for R. rickettsia, the causative agent of RMSF, since R. akari has extensive cross-reactivity. Again, a demonstration of at least a fourfold increase in antibody titers taken 2-6 weeks apart is indicative of infection.

Chikungunya (AAP, 2021c): "Laboratory diagnosis generally is accompanied by testing serum to detect virus, viral nucleic acid, or virus-specific immunoglobulin (Ig) M and neutralizing antibodies." RT-PCR can be used to diagnose chikungunya during the first week after onset of symptoms since chikungunya-specific antibodies have not formed at that time. After the first week, serum testing of IgM or a plaque-reduction neutralization test can be performed.

Dengue (AAP, 2021d): "Dengue virus is detectable by RT-PCR or NS1 antigen EIAs from the beginning of the febrile phase until day 7 to 10 after illness onset." Cross-reactivity occurs between anti-dengue virus IgM and other flaviviruses, including Zika. IgG EIA and hemagglutination testing is not specific for diagnosis of dengue, and IgG antibodies remain elevated for life; consequently, a fourfold increase in IgG between the acute and convalescent phase can confirm recent infection. "Reference testing is available from the Dengue Branch of the Centers for Disease Control and Prevention."

Malaria (AAP, 2021f): Microscopic identification of Plasmodium on both thick and thin blood films should be performed. "If initial blood smears test negative for Plasmodium species but malaria remains a possibility, the smear should be repeated every 12 to 24 hours during a 72-hour period… Serologic testing generally is not helpful, except in epidemiologic surveys… Species confirmation and antimalarial drug resistance testing are available free of charge at the Centers for Disease Control and Prevention (CDC) for all cases of malaria diagnosed in the United States." One FDA-approved RADT is available in the U.S. to hospitals and commercial labs; however, both positive and negative test results must be corroborated by microscopic examination.

West Nile Virus (WNV) (AAP, 2021i): PCR is not recommended for diagnosis of WNV in immunocompetent patients since WNV RNA is usually no longer detectable by the initial onset of symptoms. "Detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or CSF is the most common way to diagnose WNV infection." Anti-WNV IgM levels can remain elevated for longer than 1 year so a positive test result may be indicative of a prior infection. "Plaque-reduction neutralization tests can be performed to measure virus-specific neutralizing antibodies and to discriminate between cross-reacting antibodies from closely related flaviviruses. A fourfold or greater increase in virus-specific neutralizing antibodies between acute-and convalescent-phase serum specimens collected 2 or 3 weeks apart may be used to confirm recent WNV infection."

International Encephalitis Consortium (IEC)

In 2013, the IEC released their *Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis*. Concerning arboviruses, they state the following: "For most arboviruses, serologic testing of serum and CSF is preferred to molecular testing, since the peak of viremia typically occurs prior to symptom onset. For example, in patients with West Nile virus (WNV) associated with neuroinvasive disease, CSF PCR is relatively insensitive (57%) compared with detection of WNV IgM in CSF. The cumulative percentage of seropositive patients increases by approximately 10% per day during the first week of illness suggesting the need for repeat testing if the suspicion for disease is strong in those with initially negative results. Notably, arbovirus IgM antibodies may be persistently detectable in the serum and, less commonly, in the CSF, for many months after acute infection, and therefore may not be indicative of a current infection. Therefore, if possible, documentation of acute infection by seroconversion and/or 4-fold or greater rises in titre using paired sera is recommended (Venkatesan et al., 2013)."

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On 6/29/2017, the FDA approved the Rickettsia Real-Time PCR Assay (K170940) by the Centers for Disease Control and Prevention (CDC) with the following definition: "An in vitro diagnostic test for the detection of Rickettsia spp. nucleic acids in specimens from individuals with signs or symptoms of rickettsial infection and epidemiological risk factors consistent with potential exposure. Test results are used in conjunction with other diagnostic assays and clinical observations to aid in the diagnosis infection, in accordance with criteria defined by the appropriate public health authorities in the Federal government (FDA, 2018)."

On 9/1/2009, the FDA approved the BinaxNOW Malaria Positive Control Kit (K083744) rapid diagnostic test (RDT), an in vitro qualitative immunochromatographic assay, for use by hospital and commercial laboratories, but it is not approved for individual or physician offices (Arguin & Tan, 2017; FDA, 2018).

As of 8/7/2018, the FDA has approved the following assays for the detection of West Nile Virus (FDA, 2018): West Nile Virus ELISA IgG model EL0300G and West Nile Virus IgM Capture ELISA model EL0300M by Focus Technologies, Inc., West Nile Virus IgM Capture ELISA model E-WNV02M and West Nile Virus IgG Indirect ELISA by Panbio Limited, West Nile Detect IgM ELISA by Inbios Intl, Inc., Spectral West Nile

Virus IgM Status Test by Spectral Diagnostics, Inc., and the EUROIMMUN Anti-West Nile Virus ELISA (Biggs et al.) and EUROIMMUN Anti-West Nile Virus ELISA (IgM) by Euroimmun US, Inc.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86280	Hemagglutination inhibition test (HAI)
86619	Antibody; Borrelia (relapsing fever)
86666	Antibody; Ehrlichia
86750	Antibody; Plasmodium (malaria)
86753	Antibody; protozoa, not elsewhere specified
86757	Antibody; Rickettsia
86788	Antibody; West Nile virus, IgM
86789	Antibody; West Nile virus
86790	Antibody; virus, not elsewhere specified
86794	Antibody; Zika virus, IgM
87040	Culture, bacterial; blood, aerobic, with isolation and presumptive identification of isolates (includes anaerobic culture, if appropriate)
87207	Smear, primary source with interpretation; special stain for inclusion bodies or parasites (eg, malaria, coccidia, microsporidia, trypanosomes, herpes viruses)
87449	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]), qualitative or semiquantitative; multiple-step method, not otherwise specified, each organism
87662	Infectious agent detection by nucleic acid (DNA or RNA); Zika virus, amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87899	Virus isolation; centrifuge enhanced (shell vial) technique, includes identification with immunofluorescence stain, each virus
0043U	Tick-borne relapsing fever Borrelia group, antibody detection to 4 recombinant protein groups, by immunoblot, IgM Proprietary test: Tick-Borne Relapsing Fever Borrelia (TBRF) ImmunoBlots IgM Test Lab/Manufacturer: IGeneX Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes
3/22/23	Modified coverage criteria #10, 11, 14, and 15 as follows: "10) For individuals suspected of having malaria (see Note 5), the use of NAAT (including PCR) **MEETS COVERAGE CRITERIA.**; 11) For individuals suspected of having malaria (see Note 5), IFA for Plasmodium antibodies **DOES NOT MEET COVERAGE CRITERIA.**; 14) For individuals suspected of having WNV (see Note 7), the use of NAAT (including PCR) for WNV **MEETS COVERAGE CRITERIA.**; 15) For individuals suspected of having WNV (see Note 7), IFA for WNV-specific IgG antibodies in either serum or CSF **DOES NOT MEET COVERAGE CRITERIA.**"
10/27/23	The following changes were implemented: information and coverage for Zika virus testing was moved into this policy; title of policy changed to "Testing for Vector-Borne Infections" (was previously titled Testing for Mosquito- and Tick-Related Infections); due to the similarities in symptoms and the higher prevalence of Dengue than Zika, guidelines now recommend that non-pregnant, symptomatic individuals get tested for dengue and NOT for Zika; reflected in updated coverage criteria #5: "For the detection of dengue virus (DENV), the use of NAAT, IgM antibody capture ELISA (MAC-ELISA), or NS1 ELISA, as well as a confirmatory plaque reduction neutralization test for DENV, **MEETS COVERAGE CRITERIA** in the following individuals: a) For individuals suspected of having DENV (see Note 4). b) For non-pregnant individuals who are symptomatic for Zika virus infection (see Note 5)."; addition of new coverage criteria # 18-21: "18) For the detection of Zika virus, the use of NAAT **MEETS COVERAGE CRITERIA** in the following individuals:

	a) Up to 12 weeks after the onset of symptom for symptomatic (see Note 5) pregnant individuals who have either recently traveled to areas with a risk of Zika (see Note 12) or who have had sex with someone who either lives in or has recently traveled to areas with a risk of Zika (see Note 12). b) For infants born from individuals who, during pregnancy, tested positive for Zika virus. c) For infants born with signs and symptoms of congenital Zika syndrome (see Note 13) and who have a birthing parent who, during pregnancy, traveled to an area with a risk of Zika (see Note 12). 19) For pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection (see Note 13), Zika virus NAAT (maternal serum and maternal urine) and Zika virus IgM testing (maternal serum), as well as a confirmatory plaque reduction neutralization test for Zika**, MEETS COVERAGE CRITERIA.** 20) For non-pregnant individuals symptomatic for Zika virus infection (see Note 5), NAAT and/or IgM testing for Zika detection **DOES NOT MEET COVERAGE CRITERIA.** 21) For asymptomatic individuals, testing for babesiosis, chikungunya virus, CTF, DENV, ehrlichiosis and/or anaplasmosis, malaria, rickettsial disease, TBRF, WNV, YFV, or Zika virus during a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA.";** addition of *B. miyamotoi* to Note 9, causative agent of TBRF.

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AVALON HEALTHCARE SOLUTIONS

Testosterone

Policy #: AHS – G2013	**Prior Policy Name & Number (as applicable):** G2013 – Hormonal Testing in Adult Males, Testosterone Testing
Implementation Date: 9/15/21	**Date of Last Revision:** 1/19/22, 3/13/23 (See Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Testosterone is a naturally occurring lipophilic androgen hormone that is produced by both males and females for various functions. In males, testosterone is produced by the interstitial cells of Leydig in the testis. Testosterone is required for synthesis of dihydrotestosterone (DHT) as well as estradiol (E2). Sex hormone-binding globulin (SHBG) binds testosterone to aid in transport and intratesticular bioavailability.

Dysregulation in testosterone levels could lead to serious conditions, including hypogonadism and other testosterone excess or deficiency conditions. Additional hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, play roles in male development. As part of the hypothalamic-pituitary-gonadal axis, FSH and LH bind to gonadal receptors to modulate testosterone. During conditions of dyshomeostasis, such as hypogonadism, FSH, LH, and prolactin serum levels can be used as diagnostic tools (Bhasin et al., 2018; Gill-Sharma, 2018).

II. Related Policies

Policy Number	Policy Title

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

Testosterone Testing, continued

1. Measurement of serum total testosterone* (See Note 1) **MEETS COVERAGE CRITERIA** in in any of the following situations:
   a) For symptoms of androgen deficiency or androgen excess in males:
      i) For initial screening, two measurements at least 24 hours apart.
      ii) If the initial screening was normal but symptoms persist, follow-up testing is allowed no sooner than 60 days after the initial screening.
   b) For the monitoring of treatment response in men taking enzyme inhibitors for prostate cancer.
   c) For men receiving testosterone replacement therapy (every 2-3 months for the first year after initiation of therapy or after a change in therapeutic dosage; annually thereafter).
   d) For gender-dysphoric/gender-incongruent persons (baseline, during treatment, and for therapy monitoring).
   e) For symptomatic females (see Note 2) being evaluated for conditions associated with androgen excess (e.g., polycystic ovary syndrome and functional hypothalamic amenorrhea).

2. For males with total testosterone confirmed as low or borderline low and who have hypogonadism, gynecomastia, and/or other forms of testicular hypofunction, annual measurement of serum free testosterone, sex hormone-binding globulin (SHBG), and/or albumin **MEETS COVERAGE CRITERIA**.

3. For individuals suspected of having a disorder that is accompanied by increased or decreased SHBG levels (see Notes 3 and 4), measurement of serum free testosterone using a medically accepted algorithm based on total serum testosterone, SHBG, and/or albumin or bioavailable testosterone **MEETS COVERAGE CRITERIA**.

4. Prior to initiating testosterone therapy for males with gynecomastia, once per lifetime serum estradiol measurement **MEETS COVERAGE CRITERIA**.

5. For individuals with ambiguous genitalia, hypospadias, or microphallus, measurement of serum dihydrotestosterone for the diagnosis of 5-alpha reductase deficiency **MEETS COVERAGE CRITERIA.**

6. Measurement of serum free testosterone and/or bioavailable testosterone as a primary test (i.e., in the absence of prior serum total testosterone measurement) **DOES NOT MEET COVERAGE CRITERIA**.

7. For asymptomatic individuals or for individuals with non-specific symptoms, measurement of serum total testosterone, free testosterone, and/or bioavailable testosterone **DOES NOT MEET COVERAGE CRITERIA**.

8. For the identification of androgen deficiency in women, measurement of serum testosterone **DOES NOT MEET COVERAGE CRITERIA.**

Testosterone Testing, continued

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

9. The use of saliva for the measurement of testosterone **DOES NOT MEET COVERAGE CRITERIA**.

10. For all other situations not mentioned above, measurement of serum dihydrotestosterone **DOES NOT MEET COVERAGE CRITERIA**.

Notes:

Note 1: Serum total testosterone sample collection should occur in the early morning, after fasting. Due to considerable variability in serum total testosterone levels, the Centers for Disease Control and Prevention (CDC) developed a standardization program for total testosterone assays (Hormone Standardization [HoSt]/Testosterone). An assay certified by the CDC's HoSt/Testosterone program is standardized to within ±6.4% of the CDC total testosterone reference standard. It is **STRONGLY RECOMMENDED** that serum total testosterone measurement be performed with an assay that has been certified by the CDC HoSt/Testosterone program (Bhasin et al., 2018). A list of CDC-certified assays is available on the HoSt website (CDC, 2022).

Note 2: When measuring serum total testosterone in females, please note that the technology used for measurement must be sensitive enough to detect the low serum total testosterone levels that are normally found in females.

Note 3: Conditions associated with decreased SHBG concentrations according to the 2018 Endocrine Society Guidelines (Bhasin et al., 2018):

- Obesity
- Diabetes mellitus
- Use of glucocorticoids, progestins, and androgenic steroids
- Nephrotic syndrome
- Hypothyroidism
- Acromegaly
- Polymorphisms in the SHBG gene

Note 4: Conditions associated with increased SHBG concentrations according to the 2018 Endocrine Society Guidelines (Bhasin et al., 2018):

- Aging
- HIV disease
- Cirrhosis and hepatitis
- Hyperthyroidism
- Use of some anticonvulsants
- Use of estrogens
- Polymorphisms in the SHBG gene

IV. Scientific Background

The steroid hormone, testosterone, plays a role in both male and female development and health. In males, testosterone is involved in the stage-specific differentiation of germ cells, spermatogenesis, and the synthesis of dihydrotestosterone (DHT) and estradiol (E2). DHT stimulates sexual differentiation of male genitalia during embryogenesis, genital maturation during puberty, and growth of pubic and facial hair (Kinter & Anekar, 2020). E2 is required in males for modulating libido, erectile function, and spermatogenesis (Schulster et al. 2016). Serum testosterone is typically solubilized by binding to the androgen-binding protein (ABP) or sex hormone-binding globulin (SHBG), which aids in regulating their transport, distribution, metabolism, and biological activity. ABP and SHBG have similar primary structure, but they differ in the types of oligosaccharides associated with them (Hammond & Bocchinfuso, 1995).

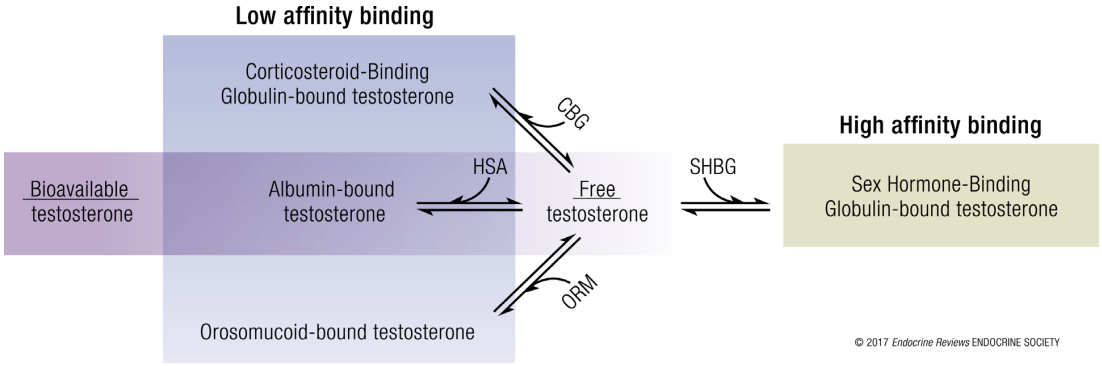
In females, testosterone is primarily synthesized and secreted in the ovaries and adrenal glands (Longcope, 1986) but some testosterone production also occurs in peripheral tissues like muscle, fat, breast, and bone (Burger, 2002). Polycystic Ovary Syndrome (PCOS) is one manifestation of a dysregulation of testosterone in women and is a complicated condition with a variety of metabolic, reproductive, and psychological features (Teede et al., 2018).

Primary and secondary hypogonadism, two forms of testicular hypofunction, can be differentiated by the concentration of serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin. Primary hypogonadism is associated with low levels of testosterone and normal to high levels of LH and FSH. Secondary hypogonadism is associated with low levels of testosterone and normal to low levels of LH and FSH (Carnegie, 2004). The anterior pituitary gland of hypothalamic-pituitary-gonadal axis releases LH and FSH, which act on the gonadal receptors to regulate testosterone production. Binding of LH to Leydig cell receptors initiates testosterone production, while testosterone secretion is further regulated by feedback inhibition (Nassar & Leslie, 2018). Males who develop hypogonadism prior to puberty often exhibit depressed secondary sex characteristics, eunuchoid stature, small testes, gynecomastia, and a small phallus. For males who develop hypogonadism after the onset of puberty, the physical findings are similar, except for a normal stature and normal phallus size (Snyder, 2020). Besides hypogonadism, testosterone production can also be affected by certain medications, chemotherapy, lifestyle, and aging (Meldrum et al., 2012; Nassar & Leslie, 2018).

In adult males, total serum testosterone levels decrease at an average rate of 1.6% per year. The concentrations of free and bioavailable testosterone decrease more rapidly, typically 2% - 3% annually, due to the natural increase in SHBG. By the age of 60, 20% of men will have testosterone levels below the normal range, "and the figure rises to 50% in those aged over 80" (Stanworth & Jones, 2008). Significant decrease in testosterone may result in symptoms such as fatigue, decreased libido, erectile dysfunction, depression, muscle weakness, and others. Unfortunately, these symptoms are not specific to testosterone deficiency (Bhasin et al., 2018). Low testosterone levels are associated with diabetes (Hassanabad & Fatehi, 2018), metabolic syndrome (Mohammed, Al-Habori, Abdullateef, & Saif-Ali, 2018), cardiovascular disease (G. Corona et al., 2018; Wang et al., 2018), obesity (Molina-Vega et al., 2018), sleep apnea (Viana et al., 2017), and other disorders (Nassar & Leslie, 2018). Additionally, testosterone elevations are associated with serious conditions including tumors, hyperthyroidism, and genetic disorders such as congenital adrenal hyperplasia (Shalender Bhasin et al., 2018; Nassar & Leslie, 2018).

Testosterone Testing, continued

Within the serum, testosterone can be either free (i.e. not bound to a specific protein) or protein-bound. Only 1% - 4% of circulating testosterone is usually found free. SHBG binds testosterone with a high affinity whereas serum albumin (HSA), corticosteroid-binding globulin (CBG), and orosomucoid binds testosterone with a much lower affinity. "Bioavailable" testosterone refers to the amount of free testosterone and albumin-bound testosterone as indicated in the figure below (Goldman et al., 2017).



CDC Hormone Standardization (HoSt) Program--Testosterone

Serum testosterone testing can measure either total testosterone (TT) concentration, free testosterone, or bioavailable testosterone. Total testosterone can be "measured using radioimmunoassay, immunometric assays, or liquid chromatography–tandem mass spectrometry. Considerable inter-assay and inter-laboratory variability is often found in TT measurements. When 1133 laboratories using 14 different assays measured TT concentrations using the same College of American Pathologists quality control sample from a single hypogonadal man, the measured values ranged from 45 to 365 ng/dL (1.6 to 12.7 nmol/L) (Bhasin et al., 2018)."

The Centers for Disease Control and Prevention (CDC) released their analysis of total testosterone in serum by isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) in 2012. As a part of the CDC HoSt Program (CDC Hormone Standardization Program) to certify and calibrate hormone assays, the CDC monitors and validates hormone testing by laboratories and manufacturers. "Calibration is further verified by analyzing serum material with assigned reference values for total testosterone every 6 months and comparing the results obtained against predefined acceptance limit, which is ± 6.4 % from the target value (CDC, 2012)." According to CDC standards, total testosterone using ID/HPLC/MS/MS methodology has a reportable range of 2.5 – 1000 ng/dL or 0.09 – 34.7 nM with a limit of detection of 0.36 ng/dL or 0.012 nM. As for accuracy in terms of "trueness and precision," the CDC reports that total serum testosterone precision (%CV) ranges from 2.2% to 5.5%. The following limitation was noted: "This method was tested for total testosterone analysis in human serum and may not be suitable for other specimens such as plasma, whole blood, urine, and/or saliva. The analytical performance parameters need to be reassessed and verified when other specimen matrices are used (CDC, 2012)." A list of the assays certified by the CDC HoSt/Testosterone program can be found on the CDC's HoSt website (CDC, 2022).

Analytical Validity

Testosterone Testing, continued

A recent study used a liquid chromatography with tandem mass spectrometry (LC-MS-MS) method to assess salivary testosterone, androstenedione, dehydroepiandrosterone, and 17-OH-progesterone. The authors state that the accuracy of this method is "between 83.0 and 106.1% for all analytes" and conclude that this "LC-MS/MS method allowed a sensitive evaluation of androgen salivary levels and represents an optimal technique to explore the relevance of a comprehensive androgen profile as measured in saliva for the study of androgen secretion modulation and activity in physiologic and pathologic states (Mezzullo et al., 2017)." However, another study compared various ELISA-based salivary testosterone assays and noted that "proportional errors between the methods calls [*sic*] for caution" as one of the four methods yielded no results due to malfunction (Andersson, Bergquist, Theodorsson, & Strom, 2017). Another study compared salivary testosterone measurements using immunoassays with those measured by tandem mass spectrometry. The authors conclude that the immunoassay-based methods "tended to inflate estimates of lower testosterone concentrations" (Welker et al., 2016).

Recently, van der Veen et al. (2019) developed and validated a LC-MS/MS method to establish reliable reference intervals in five plasma steroid hormones (progesterone, 17-hydroxyprogesterone, androstenedione, testosterone and dihydrotestosterone); these researchers utilized samples from 280 healthy male and female participants over a four-month period. Women taking oral contraceptive pills were found to have lower levels of 17-OH-progesterone and androstenedione; further, it was identified that hormonal biological variation was typically greater in women compared to men (van der Veen et al., 2019). Final conclusions stated that "The gender-specific determination of the reference intervals, together with the observation that the biological variation demonstrated a high degree of variation, allows interpretation of data on individual and group level for improved biochemical characterization of patients in clinical practice (van der Veen et al., 2019)."

Star-Weinstock and Dey (2019) developed an accurate and sensitive method to measure testosterone in hypogonadal adults and children of both genders; this quantification method utilized electrospray ionization (ESI)-LC-MS/MS and achieved a "sensitivity of 1 ng/dL from 100 µL sample volume." The authors note that two highlights of this novel method are that this sample preparation technique "includes simultaneous protein precipitation and derivatization," and that this total testosterone measurement method was certified by the CDC Hormone Standardization program (Star-Weinstock & Dey, 2019).

Sun et al. (2020) developed and validated an isotope dilution ultra-performance liquid chromatography tandem mass spectrometry method (ID-UPLC-MS/MS) to measure human serum testosterone. This method offers higher accuracy and lower variability than the traditional immunoassays, especially when measuring low testosterone levels in hypogonadism. To assess accuracy of the method, pure testosterone was added to the serum samples and the actual concentrations after two serial liquid-liquid extractions were measured. The actual concentrations were close to the female and male levels, with a recovery rate ranging from 94.32 to 108.6%. Sensitivity, specificity, and precision were also measured and met the performance criteria standards established by Clinical and Laboratory Standards Institute (Lynch, 2016) and the Hormone Standardization Program of the Center for Disease Control (Yun et al., 2012). "Moreover, the [ID-UPLC-MS/MS] method exhibited a good consistency between low and high concentrations of testosterone. In addition, the method required a simple sample preparation and a small sample volume, therefore it may be suitable for routine clinical practice" (Sun et al., 2020).

The Centers for Disease Control and Prevention (CDC) reviewed testosterone testing in 2014 in a group of 6746 participants of various age groups and both sexes (Vesper et al., 2014). The positive bias identified

by steroid analyte testing indicated that the test was measuring additional compounds (and not only the analyte in question). The authors concluded that "although technologies for steroid hormone measurement have advanced significantly, measurement variability within and across laboratories has not improved accordingly… Within-assay variability for current assays is generally high, especially at low analyte concentrations" (Vesper et al., 2014).

Testosterone and other hormones (AMH, FSH, LH, free androgen index (FAI), prolactin, estradiol) have been used for the clinical diagnosis of polycystic ovary syndrome (PCOS). In a study completed by Khashchenko et al. (2020), 130 girls with PCOS had the accuracy and specificity of hormonal testing assessed and cutoffs for the most significant hormone indicators of PCOS diagnosis in adolescents were identified. The authors found that "Levels of testosterone > 1.15 nmol/L, androstenedione > 11.45 ng/mL, and LH/FSH ratio > 1.23 also showed high sensitivity of 63.2–78.2% and specificity of 84.4–93.7% in PCOS diagnosis in the studied sample of girls." (Khashchenko et al., 2020) The combined use of either four thresholds (AMH, FAI, testosterone, androstenedione, LH/FSH ratio as previously stated) yielded a diagnostic accuracy of 90.2-91.6% in predicting PCOS in adolescents (Khashchenko et al., 2020).

Clinical Validity and Utility

Equilibrium dialysis is the gold standard for determining free serum testosterone. Unfortunately, it is technically difficult and has limited availability. Compared to other less accurate methods, it is expensive. It relies on the accuracy and precision of total testosterone determination. In equilibrium dialysis, a semipermeable membrane is used to retain the bound testosterone on one side of the membrane while the free testosterone equilibrates between the two sides. It is dependent on environmental conditions including pH, ionic strength, and temperature; in fact, steroids, such as testosterone, can bind up to 2.5 times higher at 4°C than at 37°C. One study shows that increasing the temperature from 37°C to 41°C increased the free cortisol level by approximately 80% (Goldman et al., 2017).

Immunoassays to measure free and bioavailable testosterone are inaccurate. The Endocrine Society urges the use of medically accepted algorithms that rely on TT, SHBG, and/or albumin to estimate serum free testosterone (Bhasin et al., 2018). Multiple algorithms have been published (Sartorius et al., 2009; Vermeulen et al., 1999; Zakharov et al., 2015). The recent allosteric model proposed by Zakharov and colleagues models the binding of testosterone as a multi-step, dimeric process. This allosteric model has "close correspondence with those measured using equilibrium dialysis" (Zakharov et al., 2015).

A 2017 international study comprised of multiple cohorts with healthy, non-obese males attempted to "derive standardized, age-specific reference ranges" for circulating testosterone; it was stated that "a substantial proportion of intercohort variation in testosterone levels is due to assay differences (Travison et al., 2017)." Further, the issue in developing standards for circulating testosterone due to variation in body mass and comorbidities was also noted. "Another unresolved issue relates to whether the reference sample should include only the healthy nonobese men or whether it should include the entire population of men 19 to 39 years. Obesity and comorbid conditions affect circulating total testosterone concentrations; therefore, inclusion of obese men with comorbid conditions could distort the reference ranges. Whether the reference ranges generated in nonobese men are appropriate for use in obese men deserves further investigation. Even though men with known diagnoses of conditions or diseases associated with hypogonadism were excluded, it is possible a small percentage of individuals in these cohorts may be hypogonadal (Travison et al., 2017)."

Testosterone Testing, continued

Shukla et al. (2018) organized a cross-sectional study to measure the relationship between prostatic specific antigen (PSA) and serum testosterone levels in both healthy men and men with partial androgen deficiency (PADAM); a total of 255 men participated in this study. "Mean total testosterone and serum PSA was 9.35 ± 1.33 nmol/L, 1.96 ± 0.76 ng/mL in males with PADAM and 15.30 ± 1.95 nmol/L, 1.85 ± 0.73 ng/mL respectively in males without PADAM. No significant relationship was observed between serum PSA and serum testosterone levels among healthy males irrespective of PADAM (Shukla et al., 2018)." Results from this study suggest that PSA values do not need to be adjusted "for biopsy decisions according to testosterone levels" (Shukla et al., 2018).

In a retrospective cohort study, eighty-five severely hypogonadal men were observed for changes in serum prostate specific antigen (PSA) concentrations during testosterone treatment for 18 months. The Endocrine Society clinical guidelines recommend measuring PSA in hypogonadal men over the age of 50 at three months and twelve months after starting testosterone therapy and urologic referral if serum PSA > 1.4 ng/mL above baseline or to an absolute value > 4 ng/mL (S. Bhasin et al., 2018). Studies have been performed in men with mild to moderate hypogonadism which reported smaller increases in serum PSA concentrations during testosterone treatment; however, no studies have reported serum PSA changes in response to testosterone treatment of severely hypogonadal men. In this study, testosterone treatment "increased the median serum testosterone concentration from 36 ng/dL at baseline to 395 ng/dL at 6-18 months. This treatment resulted in a median increment in PSA above

baseline of 0.70 ng/mL at 6-18 months…31% of men had increases in PSA >1.4 ng/mL; and 13% of men reached absolute PSA concentrations >4.0 ng/mL" (Sachdev, Cucchiara, & Snyder, 2020). The authors suggest that "testosterone treatment of severely hypogonadal men often increases PSA above the commonly accepted thresholds for urologic referral [and] that future clinical guidelines for the expected PSA response to testosterone replacement reflect the degree of hypogonadism" (Sachdev et al., 2020).

A total of nine years of registry data, comprised of 650 patients with hypogonadism, was analyzed to determine the impact of long-term intramuscular testosterone treatment (1000 mg every 10-12 weeks) (Zitzmann, Nieschlag, Traish, & Kliesch, 2019). Serum testosterone concentrations were found to increase "from 5.7±2.3 nmol/L to 19.4±2.8 nmol/L in men with classical hypogonadism and from 7.8±2.4 nmol/L to 19.2±3.1 nmol/L in men with functional hypogonadism"; final conclusions suggest that patients with the functional form of hypogonadism may benefit the most from testosterone treatment as "men with functional hypogonadism were more likely to lose 10% weight and 5% of waist circumference (WC) than men with classical hypogonadism" (Zitzmann et al., 2019). Men with functional hypogonadism were also more likely to be obese at the start of the study.

Cauley et al. (2021) performed a study to examine the effect of testosterone treatment on TBS. "Two hundred and eleven men were enrolled in Bone Trial of the Testosterone Trials. Of these, 197 men had 2 repeat TBS and vBMD measurements; 105 men were allocated to receive testosterone, and 92 men to placebo for 1 year. TBS, a BMD, and vBMD were assessed at baseline and month 12." The results of this study report that there was no difference in the percent change in TBS by randomized group. They saw a 1.6% (95% confidence intervals (CI) 0.2–3.9) change in the testosterone group and a 1.4% (95% CI−0.2, 3.1) change in the placebo group. In contrast, they saw a 6% increase in vBMD (95% CI4.5–7.5) in the testosterone group as compared to only a 0.4% vBMD change (95% CI−1.65–0.88) in the placebo groups (Cauley et. al 2021). As a result, the authors concluded that TBS was not clinically useful in monitoring the 1-year effect of testosterone treatment on the bone structure in older hypogonadal men (Cauley et al, 2021).

V. Guidelines and Recommendations

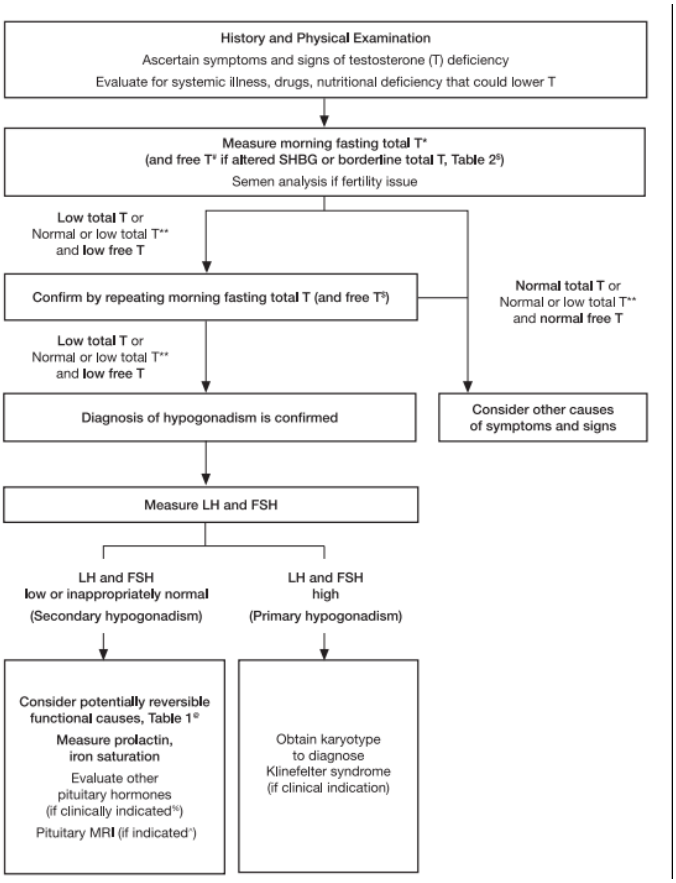
The Endocrine Society (ES)-Androgen Deficiency Testosterone Therapy

The ES, in the updated 2018 guidelines concerning testosterone therapy and hypogonadism in males, summarized their recommendations with respect to testosterone testing with the following:

- Recommendation 1.1: "We recommend diagnosing hypogonadism in men with symptoms and signs of testosterone deficiency and unequivocally and consistently low serum total testosterone and/or free testosterone concentrations (when indicated)." (Level 1+++)
- Recommendation 1.2: "We recommend against routine screening of men in the general population for hypogonadism." (Level 1++)
- Recommendation 1.3: "In men who have hypogonadism, we recommend distinguishing between primary (testicular) and secondary (pituitary–hypothalamic) hypogonadism by measuring serum luteinizing hormone and follicle-stimulating hormone concentrations." (Level 1+++)
- Recommendation 1.4: "In men with hypogonadism, we suggest further evaluation to identify the etiology of hypothalamic, pituitary, and/or testicular dysfunction." (Level 2++)
- Recommendation 3.1: "In hypogonadal men who have started testosterone therapy, we recommend evaluating the patient after treatment initiation to assess whether the patient has responded to treatment, is suffering any adverse effects, and is complying with the treatment regimen." (Ungraded Good Practice Statement)

Within the explanations and technical comments of the recommendations, the ES specifically states, "Clinicians should not use direct analog-based free testosterone immunoassays, as they are inaccurate." Moreover, recommendations state that serum total testosterone testing is preferred and should be performed on two separate days after fasting since testosterone concentrations can vary due to many circumstances. For men who initially test low total testosterone, who test near the lower limit, or who have conditions associated with altering sex hormone-binding globulin (SHBG), then free testosterone can be measured either by using an accepted algorithm based on the total testosterone, SHBG, and albumin concentrations or by direct equilibrium dialysis methods rather than the use of immunoassays. As for bioavailable testosterone testing, the ES states, "Measuring bioavailable [testosterone] concentrations using ammonium sulfate precipitation is technically challenging. Furthermore, there are no detailed studies (similar to those described previously that relate FT [free testosterone] concentrations to manifestations of [testosterone] deficiency) that use bioavailable [testosterone] concentrations." Men beginning hormone replacement therapy should have their serum testosterone and hematocrit levels measured initially to establish a baseline and then, depending on the therapy, have the levels measured again three-to-six months later. While on testosterone therapy, the total testosterone and hematocrit levels should be checked annually thereafter. Concerning secondary hypogonadism, serum prolactin and either serum ferritin or iron saturation measurements are recommended to check for the possibility of reversibility of the condition. The testing algorithm also recommends testing serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to differentiate primary and secondary hypogonadism. The algorithm for testing for hypogonadism is shown in the figure below (Bhasin et al., 2018):

Testosterone Testing, continued



The Endocrine Society – Functional Hypothalamic Amenorrhea: An Endocrine Society Clinical Practice Guideline

Testosterone testing in addition to other endocrine laboratory tests is recommended as part of an initial endocrine assessment for women with clinical hyperandrogenism in the evaluation of suspected Functional Hypothalamic Amenorrhea (FHA). Functional Hypothalamic Amenorrhea is a condition of anovulation, in which the ovary fails to release an egg during the menstrual cycle and has been correlated with stress, weight loss, and excessive exercise (Gordon et al., 2017).

The Endocrine Society (ES) - Polycystic Ovary Syndrome (PCOS)

Relative to the diagnosis of PCOS, the ES identifies three criteria that may be evaluated: androgen excess, ovulatory dysfunction, and polycystic ovaries. Two of the three criteria are sufficient for diagnosis, and if both clinical criteria are met, they do not recommend testing for androgen excess. Androgen excess is characterized by elevated serum androgen levels such as elevated total, bioavailable, or free serum testosterone levels. Considering that serum testosterone levels are variable and there is

poor standardization of assays, the Task Force recommends familiarity with local assays and does not define an absolute level that is diagnostic of PCOS or other causes of hyperandrogenism (Legro et al., 2013).

The American College of Obstetricians and Gynecologists

In 2018, the ACOG released guidelines on the clinical management of polycystic ovary syndrome (PCOS). In its suggested evaluation of patients with PCOS, the ACOG recommends having a physical, laboratory testing, and an ultrasound examination to confirm the polycystic ovaries. With regards to hormone testing, it includes "documentation of biochemical hyperandrogenemia" by "total testosterone and sex-hormone binding globulin or bioavailable and free testosterone," but notes to conduct testing that would exclude other causes of hyperandrogenism, such as thyroid dysfunction and hyperprolactinemia. ACOG includes TSH, prolactin, and 17-hydroxyprogesterone as hormones to measure to exclude other causes. The ACOG (2018b) also acknowledges that "there is no standardized testosterone assay in the United States and the sensitivity and reliability in the female ranges are often poor."

Regarding Müllerian Agenesis, ACOG writes that the initial evaluation of a patient without a uterus "may include the following laboratory tests: testosterone level, FSH level, and karyotype" (ACOG, 2018a).

In 2019, ACOG released a guideline regarding the "screening and management of the hyperandrogenic adolescent." In it, they state that the diagnosis of hyperandrogenism can be based on clinical symptoms or measurement of serum androgens. However, they recommend against monitoring serum androgens. This guideline was reaffirmed in 2020.

ACOG recommends identifying clinical symptoms of androgen excess during the initial evaluation. In the proposed algorithm for evaluation, ACOG recommends two separate batteries of hormone tests depending on type of menses. For regular menses, ACOG lists free and total testosterone, DHEAS (dehydroepianandrosterone sulphate), and 17OHP (17-α-hydroxyprogesterone) as hormones that may be tested. For irregular menses, ACOG lists prolactin, LH, FSH, TSH, and the three previously mentioned hormones. ACOG also notes that PCOS may be one of the diagnoses if both androgen excess and irregular menses are identified (ACOG, 2019).

American Urological Association (AUA)

The AUA published guidelines concerning the evaluation and management of testosterone deficiency in 2018. Five recommendations are given concerning the diagnosis of testosterone deficiency:

1. "Clinicians should use a total testosterone level below 300 ng/dL as a reasonable cut-off in support of the diagnosis of low testosterone. (Moderate Recommendation; Evidence Level: Grade B)
2. The diagnosis of low testosterone should be made only after two total testosterone measurements are taken on separate occasions with both conducted in an early morning fashion. (Strong Recommendation; Evidence Level: Grade A)
3. The clinical diagnosis of testosterone deficiency is only made when patients have low total testosterone levels combined with symptoms and/or signs. (Moderate Recommendation; Evidence Level: Grade B)

4. Clinicians should consider measuring total testosterone in patients with a history of unexplained anemia, bone density loss, diabetes, exposure to chemotherapy, exposure to testicular radiation, HIV/AIDS, chronic narcotic use, male infertility, pituitary dysfunction, and chronic corticosteroid use even in the absence of symptoms or signs associated with testosterone deficiency. (Moderate Recommendation; Evidence Level: Grade B)
5. The use of validated questionnaires is not currently recommended to either define which patients are candidates for testosterone therapy or to monitor symptom response in patients on testosterone therapy. (Conditional Recommendation; Evidence Level: Grade C)"

Other recommendations by the AUA concerning adjunctive testing in males include the following:

6. In patients with low testosterone, clinicians should measure serum luteinizing hormone levels(Strong Recommendation; Evidence Level: Grade A)
7. Serum prolactin levels should be measured in patients with low testosterone levels combined with low or low/normal luteinizing hormone levels (Strong Recommendation; Evidence Level: Grade A)
8. Patients with persistently high prolactin levels of unknown etiology should undergo evaluation for endocrine disorders (Strong Recommendation; Evidence Level: Grade A)
9. Serum estradiol should be measured in testosterone deficient patients who present with breast symptoms or gynecomastia prior to the commencement of testosterone therapy. (Expert Opinion)
10. For men undergoing testosterone therapy, "testosterone levels should be measured every 6-12 months while on testosterone therapy. (Expert Opinion)" (Mulhall et al., 2018)

The Endocrine Society (ES)-Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons

The ES published guidelines suggesting that testosterone level monitoring is suggested at baseline and every 6-12 months during suppression of puberty treatment protocol in gender-dysphoric/gender-incongruent persons. The laboratory monitoring of testosterone levels is also suggested at baseline and every 6-12 months during induction of puberty protocol. Measurement of serum testosterone levels is suggested every 3 months until levels are in the normal physiologic male range during the monitoring of transgender males on gender-affirming hormone therapy. Testosterone testing is also needed midway between injections for monitoring of testosterone enanthate/cypionate injections, alternatively peak and trough levels could be measured to ensure levels remain in the normal male range. For parenteral testosterone undecanoate, testosterone should be measured just before the following injection. For transdermal testosterone, the testosterone level can be measured no sooner than after 1 week of daily application (at least 2h after application). For monitoring transgender females on gender-affirming hormone therapy, measurement of serum testosterone is indicated every 3 months (Hembree et al., 2017, 2018a, 2018b).

American Academy of Pediatrics (AAP) —Choosing Wisely Initiative

As a part of the *Five Things Physicians and Patients Should Question* series of the Choosing Wisely initiative of the American Board of Internal Medicine (ABIM) foundation, the AAP states the following: "Avoid ordering LH and FSH and either estradiol or testosterone for children with pubic hair and/or body odor but no other signs of puberty. Premature adrenarche is usually the diagnosis and does not involve activation of the pituitary- gonadal axis but is due to an early increase in adrenal androgens. DHEA-S levels are elevated for age but do not alter the management of this common and generally benign condition (AAP, 2017)."

European Academy of Andrology (EAA)

The EAA published guidelines concerning management of bone health in males and testing in *Andrology*, a journal jointly published by the EAA and the American Society of Andrology. Recommendations include the following:

- "We recommend having serum total testosterone measured twice on a morning blood sample." (Level 1+++)
- "We recommend measuring again total testosterone and SHBG if only a single measurement documenting low testosterone is available. LH and prolactin are useful to better characterize hypogonadism." (Level 1+++)
- "We do not recommend routine measurement of serum estradiol." (Level 1++)
- "We suggest measuring estradiol only when a validated mass spectrometry-based method is available and in rare cases in which severe estrogen deficiency is suspected." (Level 2++)

Within the evidence and rationale behind the recommendations, the EAA goes on to state, "We suggest using calculated free testosterone when needed, based on the measurement of total serum testosterone, SHBG, and albumin... It can easily be obtained using online available calculators (see Appendix 2 [of (Rochira et al., 2018)] for Web links). Commercially available kits for direct measurement of free testosterone should not be used due to their poor accuracy and reliability. (Rochira et al., 2018)" Concerning other hormones, the EEA states, "all patients with documented low serum testosterone consulting with hypogonadal symptoms should receive a biochemical evaluation of their gonadal status, with measurement of serum total testosterone, SHBG, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (Rochira et al., 2018)."

The EAA also published clinical practice guidelines regarding gynecomastia evaluation and management. The EAA recommended testing several hormones for gynecomastia including "testosterone (T), estradiol (E2), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating hormone (TSH), prolactin, human chorionic gonadotropin (hCG), alpha-fetal protein (AFP), liver and renal function tests (Kanakis et al., 2019)."

Turopean Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males

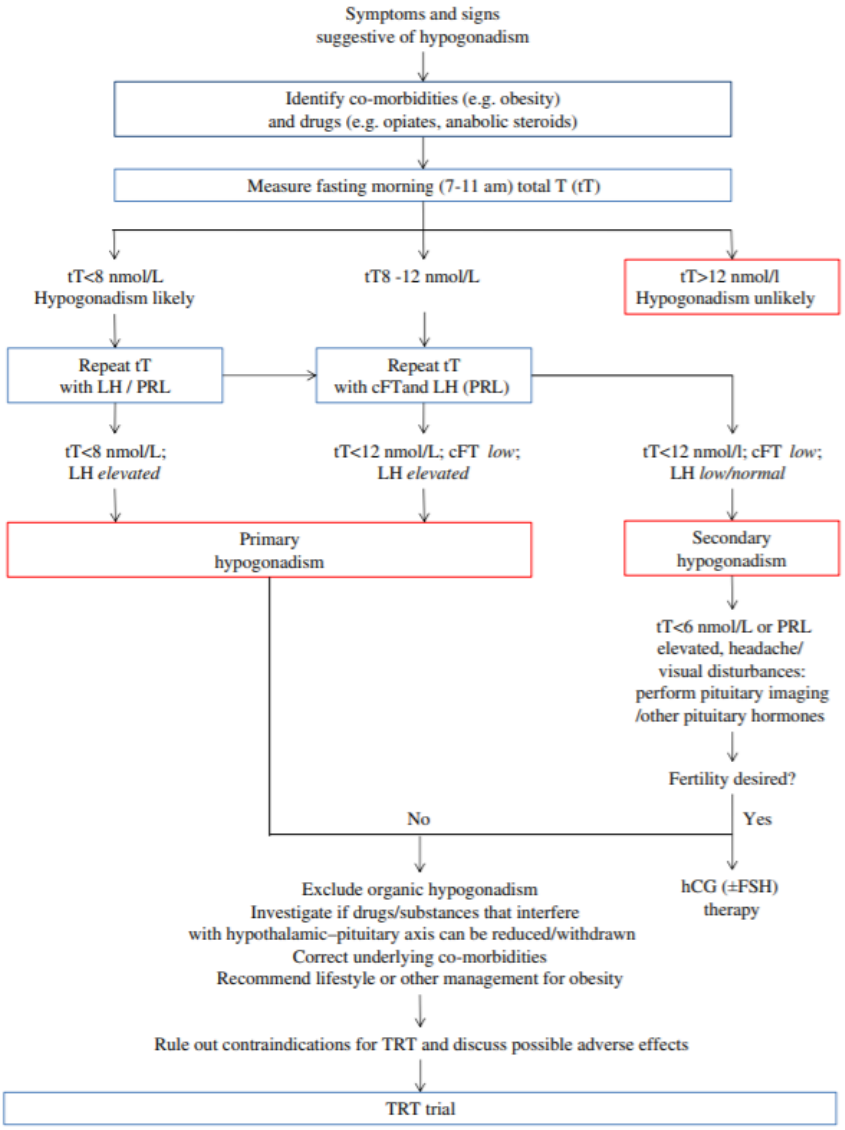
The EAA recently published clinical practice guidelines on investigation, treatment, and monitoring of functional hypogonadism in males to provide certain recommendations:

1. "We recommend against universal screening for hypogonadism in middle-aged or older men, by structured interviews or questionnaires and/or random total T measurements.

Testosterone Testing, continued

2. We recommend that the clinical diagnosis of functional hypogonadism should be confirmed by measurement of serum total T with a well validated assay on fasting morning (before 11 am) blood samples obtained on two different days.
3. Functional hypogonadism should be diagnosed only after exclusion of organic causes of hypogonadism. In addition, to morning total T, luteinizing hormone (LH) should be measured in all patients with suspected functional hypogonadism to differentiate between the primary and secondary causes.
4. We recommend either measuring or calculating free T (fT), in addition to total T, in patients with conditions that alter sex hormone-binding globulin (SHBG) and when total T concentrations are in the borderline range (~8-12 nmol/L) if the clinical suspicion of hypogonadism is strong" (Giovanni Corona et al., 2020).

Testosterone Testing, continued



Above, is a proposed flow chart to diagnose and manage functional hypogonadism (Giovanni Corona et al., 2020).

Testosterone Testing, continued

Canadian Urological Association (CUA) and Canadian Society of Endocrinology and Metabolism (CSAM)

The CUA and CSAM endorsed joint guidelines published in the *CMAJ* in 2015. The following recommendations were given concerning hormone testing in males for testosterone deficiency syndrome:

- "We recommend a thorough history and physical examination, instead of the exclusive reliance on standard questionnaires, to identify patients requiring biochemical testing (strong recommendation; moderate-quality evidence)."
- "The initial biochemical test should be total testosterone level measured in serum samples taken in the morning; determinations of bioavailable testosterone or free testosterone should be restricted to patients with equivocally low total testosterone levels (strong recommendation; high-quality evidence)."
- "We recommend that sample collection for testosterone measurement occur between 7 am and 11 am, or within 3 hours after waking in the case of shift workers (strong recommendation; moderate-quality evidence)."
- "Testosterone levels should be measured with the use of testosterone assays traceable to internationally recognized standardized reference material; commercial assays should be certified by the testosterone standardization program of the US Centers for Disease Control and Prevention (strong recommendation; high-quality evidence)."
- "Measurement of sex hormone–binding globulin with calculated free or bioavailable testosterone should be restricted to men with symptoms of testosterone deficiency and equivocally low testosterone levels (strong recommendation; moderate-quality evidence)."
- "We recommend investigation for secondary or reversible causes of hypogonadism in all men with testosterone deficiency syndrome (strong recommendation; moderate-quality evidence)."
- "We recommend investigation for testosterone deficiency syndrome and treatment with testosterone in men with anemia or sarcopenia of undetermined origin (strong recommendation; moderate-quality evidence)."
- "We recommend assessment of response and adverse effects at three and six months after onset of therapy (strong recommendation; high-quality evidence)."
- "Testosterone levels should be assessed at three and six months after onset of therapy and then annually thereafter if stable (weak recommendation; low-quality evidence)."

European Association of Urology (EAU)

In the 2014 EAU guidelines concerning the treatment of castration-resistant prostate cancer, the EAU states, "Follow-up after ADT should include analysis of PSA and testosterone levels, and screening for cardiovascular disease and metabolic syndrome (Heidenreich et al., 2014)."

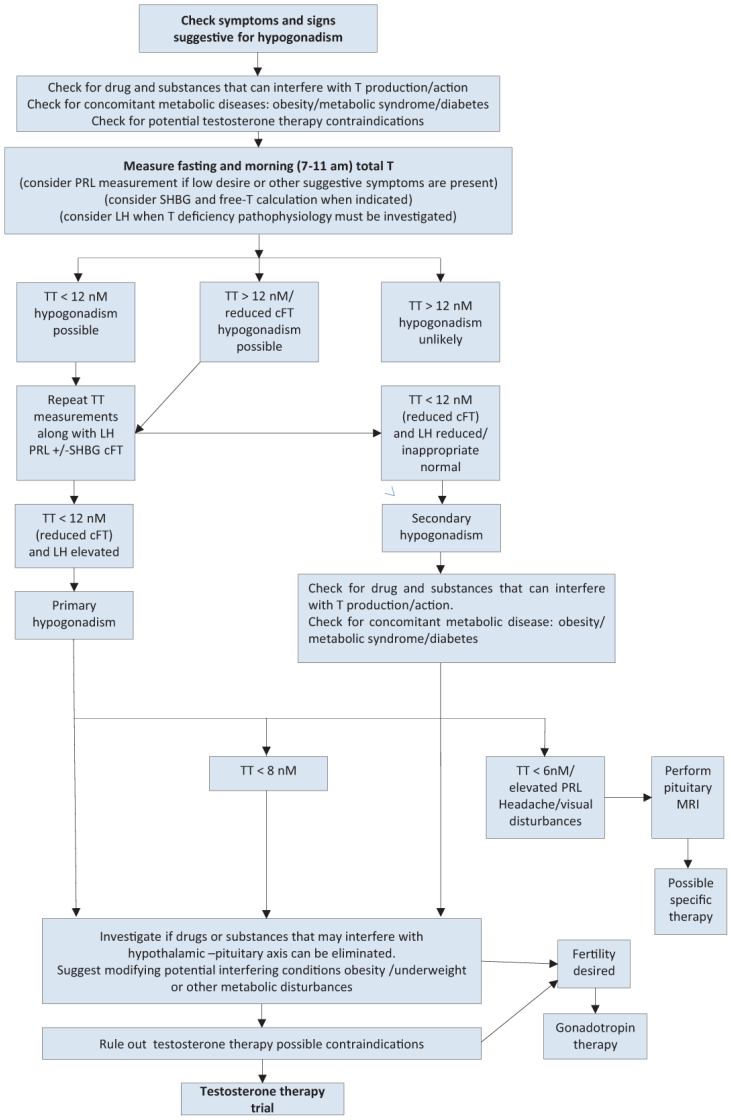
The European Association of Urology (EAU) recently released guidelines on sexual and reproductive health and expressed the following recommendations for diagnosis of hypogonadism:

Testosterone Testing, continued

1. "Check for concomitant diseases, drugs and substances that can interfere with testosterone production/action.
2. Total testosterone must be measured in the morning (7.00 and 11.00 hours) and in the fasting state, with a reliable method.
3. Repeat total testosterone on at least two separate occasions when below 12 nmol/L and before starting testosterone therapy.
4. 12 nmol/L total testosterone (3.5 ng/mL) represents a reliable threshold to diagnose late onset hypogonadism (LOH).
5. Consider sex hormone-binding globulin and free-testosterone calculation when indicated.
6. Calculated free-testosterone < 225 pmol/L has been suggested as a possible cut off for diagnosis of LOH.
7. Analyse luteinising hormone and follicle-stimulating hormone serum levels to differentiate between primary hypogonadism and secondary hypogonadism forms.
8. Consider prolactin (PRL) measurement if low desire (or other suggestive signs/symptoms) and low or low-normal testosterone is present.
9. Perform pituitary magnetic resonance imaging (MRI) in secondary hypogonadism, with elevated PRL or specific symptoms of a pituitary mass and/or presence of other anterior pituitary hormone deficiencies.
10. Perform pituitary MRI in secondary severe hypogonadism (total testosterone < 6 nmol/L).
11. Screen for late onset hypogonadism (including in T2DM) only in symptomatic men.
12. Do not use structured interviews and self-reported questionnaires for systematic screening for LOH as they have low specificity" (A. Salonia (Chair), 2020).

The EAU recommends that the standard and most accurate method for testosterone serum testing is Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Standardized automated platform immuno-assays are reliable techniques to measure testosterone; however, only LC-MS/MS can provide an accurate measurement of free testosterone (fT) levels. There is uncertainty as to what threshold of fT level indicates hypogonadism, but some data indicates that fT levels below 225 pmol/L is associated with hypogonadism. A flow chart for diagnostic evaluation of late-onset hypogonadism is provided below (A. Salonia (Chair), 2022):

Testosterone Testing, continued



National Comprehensive Cancer Network

Within the algorithm concerning the systemic therapy for castration-naïve disease, the NCCN says to "document castrate level of testosterone if on ADT [androgen deprivation therapy]" when assessing progression along with the physical exam and PSA every 3-6 months. The NCCN also states to "continue ADT [androgen deprivation therapy] to maintain castrate serum levels of testosterone (<50 ng/dL)." Additional recommendations state, "close monitoring of PSA and testosterone levels and possibly imaging is required when using intermittent ADT, especially during off-treatment periods, and patients may need to switch to continuous ADT upon signs of disease progression (NCCN, 2022b)."

The NCCN also published some guidance regarding assessment of hormones for neuroendocrine and adrenal tumors. For pituitary tumors, they list serum prolactin and LH/FSH; for "suspected or confirmed adrenocortical carcinoma", they list DHEA-S and testosterone; for hypercortisolemic Cushing's Syndrome, they list ACTH [adrenocorticotropic hormone] (NCCN, 2022a)

International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) & PanCareSurFup (PCSF) Consortium

Within the guidelines and recommendations issued in 2017 by the IGHG and the PCSF Consortium for patients with possible impaired spermatogenesis, it is recommend that "Clinical measurement of testicular volume and of follicle-stimulating hormone and inhibin B might be reasonable for the identification of impaired spermatogenesis in survivors treated with potentially gonadotoxic chemotherapy or radiotherapy potentially exposing the testes in whom semen analysis has been declined or is not possible and who desire assessment about possible future fertility. Be aware of the diagnostic limitations of these tests that may result in false positives or false negatives (level B evidence)." With respect to patients with possible testosterone deficiency, "Measurement of testosterone concentration in an early morning blood sample at clinically appropriate intervals is reasonable in post pubertal survivors treated with radiotherapy potentially exposing the testes to 12 Gy or more or with TBI (expert opinion). In the presence of clinical signs of hypogonadism, or of previous low-normal or borderline testosterone concentrations, or if it is not possible to obtain an early morning blood sample, it is reasonable to measure luteinising hormone concentration in addition to testosterone (expert opinion) (Skinner et al., 2017)."

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82040	Albumin; serum, plasma or whole blood
82642	Dihydrotestosterone (DHT)
82670	Estradiol; total
82681	Estradiol; free, direct measurement (e.g., equilibrium dialysis)
84146	Prolactin
84270	Sex hormone binding globulin (SHBG)
84402	Testosterone; free
84403	Testosterone; total
84410	Testosterone; bioavailable, direct measurement (e.g., differential precipitation)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Thyroid Disease Testing

Policy #: AHS – G2045	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 11/12/21, 4/26/22, 2/20/23, 9/27/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Thyroid hormones are necessary for both prenatal and postnatal development, as well as metabolic activity in adults (Brent, 2022).

Thyroid disease includes conditions which cause hypothyroidism, hyperthyroidism, goiter, thyroiditis (which can present as either hypo- or hyper-thyroidism) and thyroid tumors (Rugge et al., 2015).

Thyroid function tests are used in a variety of clinical settings to assess thyroid function, monitor treatment, and screen asymptomatic populations for subclinical or otherwise undiagnosed thyroid dysfunction (Ross, 2022c).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1) Thyroid function testing **MEETS COVERAGE CRITERIA** in the following situations:
   a) For individuals with symptoms consistent with hypothyroidism (see Note 1):
      i) Thyroid stimulating hormone (TSH) to confirm or rule out primary hypothyroidism.
      ii) Free T4 (fT4) as a follow up to abnormal TSH finding.

iii) TSH and fT4 in cases of suspected secondary hypothyroidism.

iv) For individuals being treated for hypothyroidism, monitoring with TSH and fT4 testing every 6-12 weeks upon dosage change and annually in stable individuals.

b) For individuals with signs and symptoms consistent with hyperthyroidism (see Note 2):

i) TSH to confirm or rule out overt hyperthyroidism.

ii) fT4 as a follow up to abnormal TSH findings.

iii) Total T3 (TT3) or free T3 (fT3) to confirm a diagnosis of hyperthyroidism.

iv) fT4 to distinguish between overt and subclinical hyperthyroidism.

v) Monitoring individuals after treatment for hyperthyroidism

(a) In patients being treated for hyperthyroidism, repeat testing of TSH and fT4 should occur every 8 weeks.

(b) Annual monitoring after first year even if asymptomatic for risk of relapse or late-onset hypothyroidism.

c) For asymptomatic individuals at high risk for thyroid disease due to:

i) A personal or family history of thyroid dysfunction.

ii) Personal or family history of type 1 diabetes or other autoimmune disorder.

iii) Prescribed drugs that can interfere with thyroid function:

(a) Annually.

(b) When dosage or medication changes.

(c) If symptoms consistent with thyroid dysfunction develop.

d) For individuals capable of becoming pregnant who:

i) Are undergoing evaluation for infertility.

ii) Have experienced two or more pregnancy losses.

e) For individuals with disease or neoplasm of the thyroid or other endocrine glands.

f) For individuals with chronic or acute urticaria.

g) For individuals undergoing immune reconstitution therapy (IRT), TSH testing:

i) Individuals with active relapsing remitting multiple sclerosis (MS) undergoing therapy with alemtuzumab (Lemtrada).

ii) Individuals with HIV undergoing highly active antiretroviral therapy (HAART).

iii) Individuals following allogeneic bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT).

h) For individuals suspected of central hypothyroidism.

i) For individuals diagnosed with primary mitochondrial disease, annual screening of TSH and fT4.

j) For pediatric individuals diagnosed with short stature.

k) For pediatric individuals with a clinical finding of failure-to-thrive.

2) For individuals who are pregnant or who are postpartum and who have symptoms of thyroid dysfunction (see Note 1 and Note 2), TSH and ft4 testing (once every 4 weeks) **MEETS COVERAGE CRITERIA (see Note 3)**.

3) For individuals who are pregnant or who are postpartum and who have been diagnosed with hyperthyroidism, total T4 (TT4), antithyroglobulin antibody (Tg-Ab), thyrotropin receptor antibodies, and anti-thyroid peroxidase antibody (TPOAb) **MEETS COVERAGE CRITERIA**.

4) For individuals with hyperthyroidism or hypothyroidism, testing for thyroid antibodies **MEETS COVERAGE CRITERIA**.

5) For individuals with thyroid cancer, testing for serum thyroglobulin and/or Tg-Ab levels for the detection of tumor recurrence, post-surgical evaluation, surveillance, and maintenance for differentiated thyroid carinomas **MEETS COVERAGE CRITERIA.**

6) For the evaluation of the cause of hyperthyroidism, testing for thyrotropin-releasing hormone (TRH) or thyroxine-binding globulin (TBG) **DOES NOT MEET COVERAGE CRITERIA**.

7) For all other situations not mentioned above, testing of reverse T3, T3 update, and TT4 **DOES NOT MEET COVERAGE CRITERIA**.

8) For the assessment of hyperthyroidism, measurement of TT3 and/or fT3 **DOES NOT MEET COVERAGE CRITERIA**.

9) To assess levothyroxine dose in hypothyroid patients, measurement of total or fT3 level **DOES NOT MEET COVERAGE CRITERIA**.

10) For asymptomatic nonpregnant individuals, testing for thyroid dysfunction during a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

Notes:

Note 1:

Signs and symptoms of hypothyroidism include:

1. Fatigue
2. Increased sensitivity to cold
3. Constipation
4. Dry skin
5. Unexplained weight gain
6. Puffy face
7. Hoarseness

8. Muscle weakness
9. Elevated blood cholesterol level
10. Muscle aches, tenderness and stiffness
11. Pain, stiffness or swelling in your joints
12. Heavier than normal or irregular menstrual periods
13. Thinning hair
14. Slowed heart rate
15. Depression
16. Impaired memory

Note 2: Hyperthyroidism can mimic other health problems, which may make it difficult for doctors to diagnose. It can also cause a wide variety of signs and symptoms, including:

1. Sudden weight loss, even when your appetite and the amount and type of food you eat remain the same or even increase
2. Rapid heartbeat (tachycardia) — commonly more than 100 beats a minute — irregular heartbeat (arrhythmia) or pounding of your heart (palpitations)
3. Increased appetite
4. Nervousness, anxiety and irritability
5. Tremor — usually a fine trembling in your hands and fingers
6. Sweating
7. Changes in menstrual patterns
8. Increased sensitivity to heat
9. Changes in bowel patterns, especially more frequent bowel movements
10. An enlarged thyroid gland (goiter), which may appear as a swelling at the base of your neck
11. Fatigue, muscle weakness
12. Difficulty sleeping
13. Skin thinning
14. Fine, brittle hair

Note 3: Due to significant changes in thyroid physiology during pregnancy, measurement of hormone levels should only be performed at labs that have trimester specific normal ranges for their assay(s). While fT4 is the preferred test, TT4 may be useful if the TSH and fT4 results are discordant or when trimester specific normal ranges for fT4 are unavailable.

III. Scientific Background

Metabolic homeostasis is regulated by the thyroid gland through production of thyroid hormones. Thyroid disease is estimated to occur in approximately 30 million Americans, much of which is undiagnosed (AACE, 2023). The thyroid gland is regulated by thyroid stimulating hormone (TSH). TSH is secreted by the anterior pituitary and stimulates the thyroid gland to secrete two hormones, thyroxine (T4) and triiodothyronine (T3), and TSH secretion is controlled through a negative feedback loop by these thyroid hormones. Thyroid function is best assessed by measuring TSH (assuming steady-state conditions and the absence of pituitary or hypothalamic disease). However, direct measurement of all TSH and all other serum thyroid hormone levels (serum total T3 and total T4, serum free T3 (fT3) and free T4 (fT4) is still important, as it may be difficult in some patients to be certain about the state of pituitary and hypothalamic function (Ross, 2022c).

Thyroid hormones must be maintained within a carefully regulated range, as levels outside this range (both hypo- or hyperthyroid) can result in adverse clinical consequences. Hypothyroidism diagnosis depends heavily on laboratory tests because of the lack of specificity of the typical clinical manifestations. Primary hypothyroidism is characterized by high TSH and low fT4 concentrations. Subclinical hypothyroidism is defined biochemically as a patient having elevated TSH but a normal fT4 concentration and secondary (central) hypothyroidism is characterized by a patient having low serum T4 concentration but a normal serum TSH concentration. Symptoms include fatigue and weakness, cold intolerance, weight gain, cognitive dysfunction, dyspnea on exertion, hair loss, hoarseness, dry skin, edema, decreased hearing, myalgia and paresthesia, depression, menorrhagia, arthralgia, or pubertal delay. (Ross, 2022a). Another well-documented consequence of hypothyroidism during childhood is that of short stature, serving as presenting feature and is linked to delayed bone age, as those treated for hypothyroidism often resume their normal growth potential (Richmond & Rogol, 2022). Thus, newborns with undetected or untreated hypothyroidism will have both mental and physical developmental delay. Hypothyroidism during pregnancy increases the risk for miscarriage, preterm delivery and pre-eclampsia (Alexander et al., 2017).

Overt hyperthyroidism refers to patients with elevated levels of fT4, fT3, or both, and subnormal TSH levels, while subclinical hyperthyroidism is defined as patients having normal T4 and T3 in the presence of subnormal TSH levels. Hyperthyroid symptoms are nonspecific, but can include tachycardia, heat intolerance, sweating, tremor, dyspnea on exertion, and weight loss. Because a number of these symptoms are so common and nonspecific, they may be subtle and unrecognized. Both hypothyroidism and hyperthyroidism conditions rely on laboratory testing to confirm diagnosis (Ross, 2022a, 2022b).

Current assays for TSH are extremely sensitive at detecting changes in thyroid homeostasis prior to changes in T4 and T3 levels. Thus, TSH assessment is the most often used initial test for thyroid function. In general, if serum TSH is normal, no further testing is needed; however, if serum TSH is high, fT4 is used to determine the degree of hypothyroidism. In contrast, if serum TSH is low, fT4 and fT3 are used to determine the degree of hyperthyroidism. If a pituitary or hypothalamic condition is suspected, both serum TSH and fT4 may be measured, and serum fT4 may be measured if symptoms of hyper- or hypothyroidism are present in a patient with normal TSH levels (Ross, 2022c). Measurement of fT4 is regarded as a better indicator of thyroid function than total T4 measurement for most situations, as it reflects the amount of available hormone. Presently there is considerable controversy as to the appropriate upper limit of normal for serum TSH, with most labs using upper limits of approximately 4.5 to 5.0 mU/L (current "normal" range 0.4-5 mU/L) and there are debates on the cost

effectiveness of screening asymptomatic patients. In addition, research has shown an age-related shift toward higher TSH concentrations in older patients (Ross, 2022c).

Thyroiditis may be caused by an autoimmune disorder, an infection, or exposure to certain drugs or toxic chemicals which can be either acute or chronic. The evaluation of possible autoimmune thyroid disorders includes testing for the presence of thyroid antibodies. Several antibodies against thyroid antigens have been described in chronic autoimmune thyroiditis. The antigens include thyroglobulin (Tg), thyroid peroxidase (TPO) and the thyrotropin receptor. Different levels of antibodies correspond to different conditions. For example, nearly all patients with Hashimoto's thyroiditis have high serum concentrations of antibodies to Tg and TPO (Ross, 2022a). Thyrotropin receptor antibodies (TRAb) can be classified as stimulating, blocking, or neutral. Stimulating TRAb cause Graves' disease; however, both blocking and stimulating antibodies can be seen in patients with Graves' disease. Although these antibodies are not typically routinely measured in evaluating thyroid function, measuring them may still be helpful for more specific goals, such as predicting progression of hypothyroidism (Ross, 2022c).

Assessment of the thyroid is particularly important for pregnant individuals. Due to the metabolic changes during pregnancy, the levels of thyroid hormones differ dramatically. In pregnant individuals, total T4 and total T3 are higher than in nonpregnant individuals, thyroxine-binding globulin nearly doubles due to the increased estrogen, and in the first trimester, TSH concentrations are reduced due to high serum human chorionic gonadotropin (hCG) levels. Thyroid physiology changes during pregnancy, therefore trimester-specific ranges for TSH and fT4 should be utilized. Unfortunately, not all commercial laboratories provide these reference ranges. As such, when trimester-specific reference ranges for fT4 are not available and fT4 levels appear discordant with TSH, total T4 measurements may be superior to fT4 (Ross, 2022d).

The effects of thyroid problems during pregnancy may be dire. Luewan, Chakkabut, and Tongsong (2011) performed a study comparing 180 pregnant women with hyperthyroidism to 360 controls. The authors found that the mean gestational age and mean birth weight were significantly lower in the study group. The incidence of fetal growth restriction, low birth weight, and preterm weights were 1.3, 1.4, and 1.3 times higher respectively in the study group compared to the control group (Luewan et al., 2011).

An imbalance of thyroid hormones is not only harmful to pregnant individuals, it can negatively impact children, producing short stature. Thyroid hormone isoforms and thyroid hormones play an important role in bone development and growth, as defects associated with congenital hypothyroidism include delayed epiphyseal closure and widely spaced cranial sutures (Leung & Brent, 2016). During development, these effects extend to influence chondrocytes and growth plate cartilage in bones. Mediation of the chondrogenesis—the formation of cartilage from condensed mesenchyme tissue—by the endocrine system takes place through the action of hormones, including growth hormone, insulin-like growth factor 1 (IGF-1), androgens, glucocorticoids, and thyroid hormone. It is believed that the balance between proliferation and senescence of chondrocytes at the growth plate of bones plays a crucial role in both normal and pathologic variations of linear growth, though the pathways are unclear as of date (Leung & Brent, 2016; Richmond & Rogol, 2022).

Tests measuring levels of thyroid-related markers are widely available, often as a panel. Many combinations of thyroid serum markers are available. For example, Testing Centers.com offer thyroid tests which screen for individual thyroid hormones including TSH, fT4, and fT3 (Testing, 2023). EverlyWell offers a direct-to-consumer home-health panel testing for TSH, T3, T4, and thyroid

peroxidase antibodies (EverlyWell, 2023). Other direct-to-consumer home-health panel tests include LetsGetChecked (LetsGetChecked, 2023), Paloma Health (Paloma_Health, 2023), (myLABBOX Thyroid Health Screening (myLABBOX, 2023), and TellmeGEN (TellmeGEN, 2023).

Common variable immunodeficiency is one of the more common antibody deficiency disorders. In one large series of primary immunodeficiency (PID) in children diagnosed over a 10-year period, CVID made up 17 of 189 total PID cases and 20 percent of the 87 cases of antibody deficiency. Most patients with CVID present after puberty, and the disorder is usually diagnosed in the second or third decade of life. However, about 25 percent of all CVID patients present in childhood or adolescence and there is an earlier peak of diagnosis occurring around eight years of age. A diagnosis of CVID before six years of age should be considered preliminary because of immunologic immaturity and the persistence of transient hypogammaglobulinemia of infancy in some children. In addition, the possible presence of a monogenic defect that causes a CVID-like disorder should be considered in children who present at a very young age. Children with failure-to-thrive should be evaluated for thyroid function and growth hormone deficiency. Growth hormone replacement therapy should be offered if deficiency is identified (Hogan, 2020).

Analytical Validity

The current generation of assays measuring serum TSH is a chemiluminometric assay, which have detection limits of about 0.01 mU/L. This amount is sufficiently low enough to distinguish between euthyroidism and hyperthyroidism as well as providing superior sensitivity to the prior generation of assays whose detection limits were approximately 0.1 mU/L (Ross, 2022c).

A study focusing on validating a new electrochemiluminescent assay for serum TSH, T4, and T3 found their intra-assay coefficient of variation to be under 8% for all three hormones and inter-assay coefficient of variation to be <2.9% for TSH, 2.3% for FT4, and 12.3% for T3. The correlation between this assay and the typical ELISA or RIA assays were all at least r = .8 with many correlations near or above .9 (Kazerouni & Amirrasouli, 2012).

Serum T4 and T3 are typically measured by automated competitive binding chemiluminometric assays. Older competitive binding radioimmunoassays are still available for serum total T4. Serum total T4 and total T3 measure both bound and unbound (free) T4 and T3, respectively. A large percentage of serum T4 is bound (99.97%) to TBG, transthyretin (also called TBPA [thyroxine0binding prealbumin]), and albumin. Serum T3 is less tightly bound to TBG and TBPA but more tightly bound to albumin than T4. Normal reference ranges do vary among laboratories; however, a typical reference range for total T4 is 4.6-11.2 mcg/dL (60-145 nmol/L) and for total T3, while more variable across laboratories even than total T4, a typical reference range is ~75-195 ng/dL (~1.1-3 nmol/L) (Ross, 2022c).

The current immunoassays used to measure T3 do not always agree with other methods. For example, a study by Masika et al. (2016) compared immunoassay methods to LC/MS/MS and found that 45% of patients classified as "normal" by immunoassay were classified as "lower than 2.5th percentile" by LC/MS/MS. The authors also noted that in patients not receiving T4, 74% of their results were below the 2.5th percentile by LC/MS/MS whereas only 21% were under that mark by immunoassay. The authors speculate that this discrepancy may be due to deionidase polymorphisms, but overall conclude that because this is a significant method to diagnose thyroid issues, accuracy of T3 measurements should be paramount (Masika et al., 2016).

The measurement of reverse T3 may not be reliable. A study by Burmeister, focused on a total of 246 patients contributing 262 reverse T3 measurements, shows an inverse linear relationship between the log of TSH and reverse T3. However, Burmeister notes that hypothyroidism may cause reverse T3 to appear normal and euthyroidism may cause reverse T3 to appear low. Furthermore, it is possible that symptoms attributed to unusual reverse T3 levels are actually caused by hypothyroidism, despite normal TSH levels. Overall, Burmeister concludes that reverse T3 cannot differentiate between hypothyroidism and euthyroidism (Burmeister, 1995; Gomes-Lima & Burman, 2018).

Clinical Utility and Validity

Li et al. (2017) conducted a preliminary study to investigate how certain dietary supplements could affect clinical assays. They examined 6 healthy adult participants and 11 hormone and nonhormone analytes measured by 37 immunoassays and found that ingesting 10 mg/d of biotin for 1 week was associated with a potentially clinically important interference with some biotinylated assays. These immunoassays use a biotin-strepavidin binding system, so excess biotin may influence the results of assays using this system. The time at which the biotin was ingested was also a factor in the magnitude of the distortion (Li et al., 2017). Repeating a thyroid test at least two days after biotin discontinuation may be considered (Ross, 2022c).

Livingston, et al. (2015) assessed the impact of T3 testing and whether T3 testing provides clinically useful information to patients who are over-treated for hypothyroidism with levothyroxine. Out of 542 patients, 33 were placed in an over-treated group, 236 were placed in a control group, and the remaining 273 did not fulfill either group. None of the patients in the over-treated group had an increased T3, and the "most discriminant" T3 level was only at 58% sensitivity and 71% specificity. The authors concluded there is no reason to measure T3 in patients with hypothyroidism on levothyroixine therapy (Livingston et al., 2015).

Yazici, et al. (2016) assessed three predictors of thyroid cancer, thyrotropin (TSH), thyroglobulin (Tg), and their ratio. A study of 242 patients (134 with benign thyroid conditions, 68 with malignancy) was performed. The authors found that preoperative Tg levels were significantly lower in the malignant group (64 ng/mL vs 20 ng/mL) and that the TSH to Tg ratio was significantly higher in the malignant group, as there was no major difference in TSH between groups despite the Tg changes. However, the authors note that a multivariate analysis revealed only fine-needle aspiration biopsy was a significant factor (Yazici et al., 2016). Autoantibodies may also play a role in the diagnosis of cancer. A study by Gholve assessing 301 samples from differentiated thyroid cancer patients (compared to 37 euthyroid controls) found the prevalence of autoantibodies in the cancer patients to be significantly higher than the controls. The authors found the prevalence of the antibodies to be 17.3% by the Immunotech kit and 16.6% by the radioassay in patients with cancer, whereas the control group was found to be only 5.4% by both methods (Gholv et al., 2017).

Thyroid antibodies play a role in autoimmune thyroiditis. A study performed by Biktagirova et al. (2016) found that 97% of patients with autoimmune thyroiditis had a high antibody to denatured DNA ratio compared to healthy controls. Most of these patients also had a thyroid condition (euthyroidism, hypothyroidism, hyperthyroidism) (Biktagirova et al., 2016). Another study performed by Diana investigated the prevalence of thyroid stimulating hormone receptor (TSHR) blocking antibodies (TBAb) in autoimmune thyroid disease. 1079 patients with autoimmune thyroid disease (AITD) were compared to 302 controls.

The authors found that about 10% of patients with AITD were positive for TBAb (82/1079). TBAb also correlated positively with TSHR binding inhibiting immunoglobulins and negatively with TSHR stimulatory antibodies. The authors concluded that TBAb was a useful and important tool to identify hypothyroidism (Diana et al., 2017).

Kluesner et al. (2018) analyzed current thyroid function test ordering practices. The authors examined 38,214 tests (encompassing TSH, FT4, TSH + FT4, FT3, Total T4, and total T3). Overall, TSH alone comprised 52.14% of tests, TSH + FT4 26.72%, FT3 alone 10.63%, FT4 alone 4.26%, and TSH + FT4 + FT3 2.74%. Free thyroid hormone testing amounted to 36% of all tests. The authors estimated the annual cost of free thyroid hormone testing to be $107,720, with savings of up to $120,000 (Kluesner et al., 2018).

Jin (2018) investigated the prevalence of subclinical hypothyroidism in obese children and its association with thyroid hormone. The study included 1,104 children and 27 of 111 (24.3%) obese children were found to have subclinical hypothyroidism, compared to 127 of 993 (12.8%) non-obese children. Body mass index was found to positively correlate with serum concentrations of TSH and negatively correlate with serum concentrations of fT4. Total cholesterol and triglyceride concentration were found to positively correlate with TSH concentrations, with fT4 negatively correlating with total cholesterol. Jin concluded that TSH is correlated with lipid profiles (Jin, 2018).

In a 2018 study, Muraresku et al. (2018) reviewed mitochondrial disease and recent advances in clinical diagnosis, management, therapeutic development, and preventative strategies. They noted that routine screening of individuals with mitochondrial diseases is imperative. Screening should include examining the "multitude of symptoms known for diabetes mellitus, adrenal insufficiency, thyroid hormone insufficiency, hearing loss, cardiac arrhythmias, and other disease related symptoms, with appropriate multi-specialist management provided." They also noted that "primary mitochondrial disease encompasses an impressive range of inherited energy deficiency disorders having highly variable molecular etiologies as well as clinical onset, severity, progression, and response to therapies of multi-system manifestations" (Muraresku et al., 2018).

Sarkar (2012) examined literature surrounding recurrent pregnancy loss in patients with thyroid dysfunction. Disturbances in thyroid function and thyroid hormone levels are common in women during their reproductive years and that dysfunction can interfere with reproductive physiology, can reduce the likelihood of pregnancy, and can adversely affect pregnancy outcome. They note that "universal screening for thyroid hormone abnormalities should be conducted in females with fetal loss or menstrual disturbances. Practitioners providing health care for women should be alert to thyroid disorders as an underlying etiology for recurrent pregnancy loss." However, universal screening for thyroid hormone abnormalities is not routinely recommended at present. In individuals capable of pregnancy and of reproductive age, hypothyroidism can be reversed by thyroxine therapy and this can improve fertility and help individuals avoid needing to use assisted reproduction technologies (Sarkar, 2012).

Korevaar et al. (2019) performed a meta-analysis focusing on thyroid function test abnormalities and thyroid autoimmunity with preterm birth. They assessed 19 cohorts encompassing 47,045 pregnant individuals and found1,234 of these women had subclinical hypothyroidism. 904 had isolated hypothyroxinemia ("decreased fT4 concentration with normal thyrotropin concentration"). 3,043 were thyroid peroxidase (TPO) antibody positive, and 2,357 had preterm birth. Risk of preterm birth was

found to be higher for individuals with subclinical hypothyroidism than with euthyroidism (odds ratio = 1.29) as well as higher for individuals with isolated hypothyroxinemia (odds ratio = 1.46). The authors also found that a one standard deviation increase in maternal serum thyrotropin concentration increased risk of preterm birth by an odds ratio of 1.04. Finally, TPO antibody positive individuals were found to have a higher risk of preterm birth compared to TPO antibody negative individuals by an odds ratio of 1.33 (Korevaar et al., 2019).

In a population-based study by Kiel et al. (2020), the use of thyroid hormone measurements in ambulatory care was assessed. Measurement of serum TSH, fT3, and fT4 within the 1-3 years prior to the study was reported. A total of 5,552 participants were included in the analysis, with 25% (1,409/5,552) having a diagnosed thyroid disorder or treatment. 30% (1626/5552) received at least one TSH measurement and 6.8% (378/5552) received at least one thyroid ultrasound. In the study, "TSH measurement rates were 1.7 times higher than the highest reported rate (438/1000), fT4 measurement rates were within the reported range (89/1000) and fT3 was measured at a 10- fold higher rate than the highest reported (89/1000)." The study results are in accordance with current guidelines, which recommend measuring TSH levels rather than fT4/fT3 both for patients with suspected hypo- and hyperthyroidism as well as for monitoring purposes. However, the data also suggests that fT4 and fT3 were tested at the same rate, even though fT4 is recommended as sufficient to distinguish between overt and subclinical hypothyroidism. Despite overuse of thyroid hormone testing, there is possible underuse in patients with diagnosed thyroid disorders who are taking thyroid medication. In the study, 40% did not receive a monitoring TSH test within 1 year, and 16% did not receive a TSH test within 3 years. The authors suggest that "Given the frequency of patients with thyroid disorders, diagnostic and monitoring tests should be used rationally with regard to costs. TSH levels should be monitored regularly in patients on thyroid medication" (Kiel et al., 2020).

In 2021, Degrandi et al. (2021) examined the prevalence of thyroid autoimmunity in children with developmental dyslexia. Serum TSH, fT3, and fT4 were measured and thyroid autoimmunity was evaluated by measuring TPOAbs and antithyroglobulin antibodies (TG-Abs). The authors also performed thyroid ultrasonography in the subjects with developmental dyslexia. The study enrolled 51 subjects with developmental dyslexia (M : F = 39 : 12, mean age 12.4 ± 9 years) and 34 controls (M : F = 24 : 10, mean age 10.8 ± 4 years) and found a significant increase in TPOAb positivity in subjects as compared to controls (60.8% vs 2.9%, $p<0.001$) but no significant change in TG-Ab positivity (16% vs 5.8%). Additionally, in the subjects with developmental dyslexia who received ultrasonography (49 of 51 subjects), 60% of them had a thyroiditis pattern. Overall, this study showed a high prevalence of thyroid autoimmunity in children with developmental dyslexia and while further research is needed to confirm these initial findings, these results may change the approach to developmental dyslexia and eventually lead to a systematic determination of thyroid autoimmunity in affected children (Degrandi et al., 2021).

Wang et al. (2021) examined the association between thyroid function and serum lipid metabolism, utilizing a genetic analysis termed Mendelian randomization (MR). While thyroid dysfunction is known to be associated with cardiovascular disease, the role of thyroid function in lipid metabolism is still partly unknown. "The MR approach uses a genetic variant as the instrumental variable in epidemiological studies to mimic a randomized controlled trial" and for this study, the authors performed a two-sample MR to assess the causal association, using summary statistics from the Atrial Fibrillation Genetics Consortium (n = 537,409) and the Global Lipids Genetics Consortium (n = 188,577). TSH, fT3 and fT4 levels, the fT3:fT4 ratio, and the concentration of TPOAb were all used to get a clinical

measurement of thyroid function. Serum lipid metabolism traits included total cholesterol (TC) and triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. To assess the association between thyroid function and serum lipid metabolism, the MR estimate, and MR inverse variance-weighted method were used. The authors found that increased TSH levels were significantly associated with higher TC and LDL levels, as was the fT3:fT4 ratio. However, they observed no significant differences between genetically predicted fT4 and TPOAb and serum lipids. They concluded that their results suggest an association between thyroid function and serum lipid metabolism, "highlighting the importance of the pituitary-thyroid-cardiac axis in dyslipidemia susceptibility" (Wang et al., 2021)

Toloza et al. (2022) performed a systematic review and meta-analyses of data collected from pregnant patients (excluding pre-existing thyroid disease and multifetal pregnancies) to analyze the primary outcomes of gestational hypertension and pre-eclampsia; data was taken from cohort studies that included maternal concentrations of TSH, FT4, and TPO antibodies as well as data regarding maternal gestational hypertension, pre-eclampsia, or both. The study comprised 46,528 pregnant individuals, of which 39,826 individuals had enough data to be classified by thyroid function status. Individuals who had subclinical hypothyroidism made up 3.2% of the cohort (1,275 individuals). After analyses, a total of 933 individuals had isolated hypothyroxinemia, 619 had subclinical hyperthyroidism, and 337 had overt hyperthyroidism. The authors concluded that "compared with euthyroidism, subclinical hypothyroidism was associated with a higher risk of pre-eclampsia…In a continuous analysis, both a higher and a lower TSH concentration were associated with a higher risk of pre-eclampsia" (Toloza et al., 2022).

IV. Guidelines and Recommendations

United States Preventive Services Task Force (USPSTF)

The USPSTF states that "current evidence is insufficient to assess the balance of benefits and harms of screening for thyroid dysfunction in non-pregnant, asymptomatic adults" (Rugge et al., 2015). In addition, USPSTF recommends against screening for thyroid cancer in asymptomatic adults (USPSTF, 2017).

American College of Obstetricians and Gynecologists (ACOG)

ACOG published an updated guideline regarding Thyroid Disease in Pregnancy in June 2020. The following recommendations are based on good and consistent scientific evidence (Level A):

- "Universal screening for thyroid disease in pregnancy is not recommended because identification and treatment of maternal subclinical hypothyroidism has not been shown to result in improved pregnancy outcomes and neurocognitive function in offspring.
- If indicated, the first-line screening test to assess thyroid status should be measurement of the TSH level.
- The TSH level should be monitored in pregnant women being treated for hypothyroidism, and the dose of levothyroxine should be adjusted accordingly with a goal TSH level between the lower limit of the reference range and 2.5 milliunits/L. Thyroid-stimulating hormone typically is evaluated every 4–6 weeks while adjusting medications.

- Pregnant women with overt hypothyroidism should be treated with adequate thyroid hormone replacement to minimize the risk of adverse outcomes.
- The level of free T4 should be monitored in pregnant women being treated for hyperthyroidism, and the dose of antithyroid drug (thioamide) should be adjusted accordingly to achieve a free T4 at the upper end of the normal pregnancy range. Among women who also have T3 thyrotoxicosis, total T3 should be monitored with a goal level at the upper end of normal pregnancy range.
- Pregnant women with overt hyperthyroidism should be treated with antithyroid drugs (thioamides)."

The following recommendation is based on limited or inconsistent scientific evidence (Level B):

- "Either propylthiouracil or methimazole, both thioamides, can be used to treat pregnant women with overt hyperthyroidism. The choice of medication is dependent on trimester of pregnancy, response to prior therapy, and whether the thyrotoxicosis is predominantly T4 or T3."

The following recommendations are based primarily on consensus and expert opinion (Level C):

- "Indicated testing of thyroid function should be performed in women with a personal or family history of thyroid disease, type 1 diabetes mellitus, or clinical suspicion of thyroid disease.
- Measurements of thyroid function are not recommended in patients with hyperemesis gravidarum unless other signs of overt hyperthyroidism are evident."

Other miscellaneous, relevant comments from ACOG include:

- "Indicated testing of thyroid function should be performed in women with a personal or family history of thyroid disease, type 1 diabetes mellitus, or clinical suspicion of thyroid disease… In a pregnant woman with a significant goiter or with distinct thyroid nodules, thyroid function studies are appropriate…"
- "In cases of suspected hyperthyroidism, total T3 also is measured…Total T3 is used preferentially over free T3 because assays for estimating free T3 are less robust than those measuring free T4…"
- "Routine testing for antithyroid peroxidase antibodies in women who are euthyroid (e.g., no history of thyroid disease and normal thyroid function tests) is not recommended because thyroid hormone replacement for antithyroid peroxidase antibodies alone has not been found to improve pregnancy outcomes… Identification of thyroid antibodies including thyroid receptor antibodies and thyroid stimulating immunoglobulin in women with Graves disease may establish those at an increased risk for fetal or neonatal hyperthyroidism." (ACOG, 2020)

American Thyroid Association (ATA) and American Association of Clinical Endocrinologists (AACE)

The ATA and AACE support TSH testing for individuals with the following conditions: adrenal insufficiency, alopecia, unexplained anemia, unexplained cardiac dysrhythmia, skin texture changes, congestive heart failure, constipation, dementia, type 1 diabetes, dysmenorrhea, hypercholesterolemia, hypertension, mixed hyperlipidemia, malaise and fatigue, unexplained

myopathy, prolonged QT interval, vitiligo, or weight gain. The guidelines also recommend assessing serum FT4 instead of total T4 to diagnose hypothyroidism except with pregnant patients.

The ATA and AACE also provide recommendations for thyroid antibody testing including:

- "Anti-thyroid peroxidase antibody (TPOAb) measurements should be considered when evaluating patients with subclinical hypothyroidism."
- TPOAb measurement should be considered in evaluation of patients with recurrent miscarriage, regardless of fertility.
- "Measurement of TSHRAbs should be considered in hypothyroid pregnant patients with history of Graves' disease if treated with radioactive iodine or thyroidectomy before pregnancy. This should be done either at 20-26 weeks of gestation or during the first trimester and if they are elevated, again at 20-26 weeks of gestation (Garber et al., 2012)."

The guidelines recommend against testing serum T3 or free T3, as well as use of clinical scoring systems to diagnose hypothyroidism. In patients with central hypothyroidism, the guidelines recommend assessing either fT4 or its index and to avoid testing for TSH (Garber et al., 2012).

American Thyroid Association (ATA)

Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum

In 2011, the ATA stated that it does not recommend "universal" TSH or free T4 screening of pregnant women or during the preconception period. It also included the following recommendations:

Thyroid Function Tests in Pregnancy: Trimester-specific reference ranges for TSH, as defined in populations with optimal iodine intake, should be applied. The ATA recommends these reference ranges: first trimester, 0.1–2.5 mIU/L; second trimester, 0.2–3.0 mIU/L; third trimester, 0.3–3.0 mIU/L.

The best method to assess serum fT4 during pregnancy is measurement of T4 in the dialysate or ultrafiltrate of serum samples employing LC/MS/MS. If this is not available, clinicians should use the next best method available. However, serum TSH is a more accurate indication of thyroid status in pregnancy than any of these alternative methods. Method-specific and trimester-specific reference ranges of serum FT4 are required.

Thyrotoxicosis in Pregnancy: If the first trimester serum TSH appears low (<0.1 mIU/L), a history and physical examination are indicated. FT4 measurements should be obtained in all patients. Measurement of serum total T3 (TT3) and thyrotropin receptor antibodies (TRAb) may be helpful in establishing a diagnosis of hyperthyroidism. If the patient has a history of Graves' disease, a maternal serum sample of TRAb should be obtained at 20–24 weeks gestation.

Thyroid Nodules and Thyroid Cancer: Treatment of thyroid nodules during pregnancy will depend on risk assessment. However, all women should have the following: a complete history and clinical examination, serum TSH testing, and ultrasound of the neck. Thyroid hormone therapy may be considered in pregnant women who have deferred surgery for well-differentiated thyroid carcinoma until postpartum. The goal of levothyroxine (LT4) therapy is a serum TSH level of 0.1–1.5 mIU/L. Furthermore, a preconception TSH goal (determined by risk assessment) should be set in women with differentiated thyroid cancer. This goal should be maintained during pregnancy with

monitoring every 4 weeks until 16-20 weeks of gestation followed by once between 26 and 32 weeks of gestation.

Postpartum Thyroiditis (PPT): Women with postpartum depression should have TSH, FT4, and TPOAb tests performed. Women who are symptomatic with hypothyroidism in PPT should either have their TSH level retested in 4–8 weeks or be started on LT4 in certain situations (such as if symptoms are severe). Women who are asymptomatic with hypothyroidism in PPT should have their TSH level retested in 4–8 weeks. Finally, women with a history of PPT should have an annual TSH test to evaluate for permanent hypothyroidism.

Thyroid Function Screening in Pregnancy: There is insufficient evidence regarding universal TSH screening at the first trimester visit. Serum TSH values should be obtained early in pregnancy in the following women at high risk for overt hypothyroidism:

- History of thyroid dysfunction or prior thyroid surgery
- Age > 30 years
- Symptoms of thyroid dysfunction or the presence of goiter
- TPOAb positivity
- Type 1 diabetes or other autoimmune disorders
- History of miscarriage or preterm delivery
- History of head or neck radiation
- Family history of thyroid dysfunction
- Morbid obesity (BMI ≥40 kg/m^2)
- Use of amiodarone or lithium, or recent administration of iodinated radiologic contrast
- Infertility
- Residing in an area of known moderate to severe iodine insufficiency (Stagnaro-Green et al., 2011)

The ATA published an update in 2017 for thyroid function testing during pregnancy. Recommendations include:

- Total T4 measurement (with a pregnancy-adjusted reference range) is reliable for estimating concentration late in pregnancy. A free thyroxine index can also estimate FT4 well.
- Euthyroid and TPO or Tg antibody positive pregnant women should have serum TSH concentration measured at the start of pregnancy and every 4 weeks through mid-pregnancy.
- All women seeking care for infertility are recommended to have serum TSH levels measured.
- Pregnant women with TSH concentrations >2.5 mU/L should be evaluated for TPO antibodies.
- Women with hypothyroidism or those at risk for hypothyroidism (e.g. patients who are euthyroid but TPO or TGAb positive) should be monitored with a serum TSH measurement every 4 weeks until mid-gestation, and at least once near 30 weeks.
- "When a suppressed serum TSH is detected in the first trimester (TSH less than the reference range), a medical history, physical examination, and measurement of maternal serum FT4 or TT4

concentrations should be performed. Measurement of TRAb and maternal TT3 may prove helpful in clarifying the etiology of thyrotoxicosis"

- In women being treated with antithyroid drugs [ATDs] in pregnancy, FT4/TT4 and TSH should be monitored every 4 weeks.
- "All patients with depression, including postpartum depression, should be screened for thyroid dysfunction."
- "Evaluation of serum TSH concentration is recommended for all women seeking care for infertility."
- "If the patient has a past history of GD [Graves Disease] treated with ablation (radioiodine or surgery), a maternal serum determination of TRAb is recommended at initial thyroid function testing during early pregnancy. If maternal TRAb concentration is elevated in early pregnancy, repeat testing should occur at weeks 18–22.
- "If the patient requires treatment with ATDs for GD through mid-pregnancy, a repeat determination of TRAb is again recommended at weeks 18–22. If elevated TRAb is detected at weeks 18–22 or the mother is taking ATD in the third trimester, a TRAb measurement should again be performed in late pregnancy (weeks 30–34) to evaluate the need for neonatal and postnatal monitoring."
- "The utility of measuring calcitonin in pregnant women with thyroid nodules is unknown. The task force cannot recommend for or against routine measurement of serum calcitonin in pregnant women with thyroid nodules."
- "All newborns should be screened for hypothyroidism by blood spot analysis typically 2–5 days after birth."
- "Following the resolution of the thyrotoxic phase of PPT, serum TSH should be measured in approximately 4–8 weeks (or if new symptoms develop) to screen for the hypothyroid phase."
- "Women with a prior history of PPT should have TSH testing annually to evaluate for the development of permanent hypothyroidism"
- "There is insufficient evidence to recommend for or against universal screening for abnormal TSH concentrations in early pregnancy.
- "There is insufficient evidence to recommend for or against universal screening for abnormal TSH concentrations preconception, with the exception of women planning assisted reproduction or those known to have TPOAb positivity"
- "Universal screening to detect low FT4 concentrations in pregnant women is not recommended." (Alexander et al., 2017).

The guideline also lists certain populations of pregnant women that should have serum TSH measured "as soon as pregnancy is confirmed" due to presence of risk factors of thyroid disease. These risk factors include "history of thyroid dysfunction, symptoms or signs of thyroid dysfunction, presence of a goiter, and known thyroid antibody positivity…age >30 years, history of diabetes mellitus type 1, or other autoimmune disorders, history of pregnancy loss, preterm delivery or infertility, history of head or neck radiation or prior thyroid surgery, family history of autoimmune thyroid disease or thyroid dysfunction, morbid obesity, use of amiodarone, lithium, or recent administration of iodinated

radiologic contrast, two or more prior pregnancies, and residing in area of moderate to severe iodine deficiency." (Alexander et al., 2017)

ATA, *Task Force on Thyroid Hormone Replacement for Hypothyroidism Treatment* (2014)

The ATA recommended LT4 as the primary treatment of choice for hypothyroidism due to overall efficacy, low cost, and lack of side effects. The ATA also states that great care should be taken to monitor dose diligently especially in pregnant women, as excessive LT4 can have dangerous side effects (Jonklaas et al., 2014).

ATA, *Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis* (2016)

The ATA recommends that the cause of the thyrotoxicosis should be determined. Initial diagnostic tests include measurement of TRAb, radioactive iodine uptake, or measurement of thyroidal blood flow on ultrasonography. The guidelines also note that serum TSH is the most accurate and should be the first screening test done, but if thyrotoxicosis is suspected, it is helpful to test FT4 and T3.

The ATA recommends treatment of subclinical hyperthyroidism (persistent TSH <0.1 mU/L) for the high-risk populations such as those with cardiac risk factors or those older than 65. Treatment of asymptomatic and otherwise healthy individuals may be considered. The ATA also recommends testing TRAb in pregnant women with unknown hyperthyroidism. A diagnosis of hyperthyroidism should made with the serum TSH values and trimester-specific reference ranges for T4 and T3 (Ross et al., 2016).

American Academy of Family Physicians (AAFP)

The AAFP has recommended this diagnostic workup for hyperthyroidism: "measuring TSH, free (T4), and total T3 levels to determine the presence and severity of the condition, as well as radioactive iodine uptake and scan of the thyroid to determine the cause." The level of this evidence is C which is a consensus, disease-oriented evidence, usual practice, expert opinion, or case series (Kravets, 2016). The AAFP also recommends using TSH testing to diagnose primary hypothyroidism (Level C) (AAFP, 2012).

In the case of subclinical thyroid disease, the AAFP recommends that "Physicians should not routinely screen for subclinical thyroid disease" (Donangelo & Suh, 2017). Moreover, the AAFP reaffirms its support for the USPSTF stance on thyroid dysfunction, stating that there is no evidence that population screening is beneficial and that "Screening for thyroid dysfunction in nonpregnant, asymptomatic individuals has uncertain risks and benefits" as there has been a dearth of studies comparing the benefits of harms of screening against no screening (AAFP, 2018; Wilson et al., 2021).

American Academy of Pediatrics-Section on Endocrinology)

The American Academy of Pediatrics recommends against routinely measuring thyroid function or insulin levels in obese children, as well as screening healthy children for thyroid problems (AAP, 2017).

American Thyroid Association

The American Thyroid Association recommends that "the appropriate management of abnormal maternal thyroid tests attributable to gestational transient thyrotoxicosis and/or hyperemesis gravidarum includes supportive therapy, management of dehydration, and hospitalization if needed. Antithyroid drugs are not recommended, though β-blockers may be considered. In women being treated

with antithyroid drugs in pregnancy, FT4/TT4 and thyroid hormone secretion should be monitored approximately every 4 weeks. Antithyroid medication during pregnancy should be administered at the lowest effective dose of MMI or PTU, targeting maternal serum FT4/TT4 at the upper limit or moderately above the reference range. A combination regimen of LT4 and antithyroid drugs should not be used in pregnancy, except in the rare situation of isolated fetal hyperthyroidism" (Alexander et al., 2017).

Australian Journal of General Practice

The Australian Journal of General Practice recommends "couples with two of more pregnancy losses should have thyroid antibody and function testing performed. Abnormal results should be managed by a specialized clinic. There is evidence that suggests hypothyroidism and even subclinical hypothyroidism is associated with recurrent pregnancy loss. All guidelines recommend testing for thyroid-stimulating hormone (TSH) levels, but there is contention about what is considered a 'normal' TSH. Current guidelines suggest treating all women with overt hypothyroidism, considering treatment of subclinical hypothyroidism, and not treating euthyroid patients with recurrent pregnancy loss who test positive for thyroid antibodies" (Hong Li & Marren, 2018).

Joint Task Force on Practice Parameters (JTFPP) of the Academy of Allergy, Asthma & Immunology (AAAAI); the American College of Allergy, Asthma & Immunology (ACAAI); and the Joint Council of Allergy, Asthma & Immunology

The JTFPP within their guidelines concerning the diagnosis and management of acute and chronic urticaria state, "Targeted laboratory testing based on history or physical examination findings is appropriate, and limited laboratory testing can be obtained. Limited laboratory testing includes a CBC with differential, sedimentation rate, and/or C-reactive protein, liver enzyme, and thyroid-stimulating hormone (TSH) measurement… Targeted laboratory testing based on history and/or physical examination (e.g., obtaining TSH in a patient with weight gain, heat/cold intolerance, and thyromegaly) is recommended (Bernstein et al., 2014)."

American Society for Clinical Pathology (ASCP)

The American Society for Clinical Pathology recommends against ordering multiple tests for an initial evaluation for a patient with a suspected thyroid condition. The ASCP recommends starting with TSH and proceeding from that result (ASCP, 2015).

On September 1, 2020, ASCP released a recommendation to avoid TSH screening in annual well-visits for asymptomatic adults, regardless of age, as there is no evidence to support that routine screening improves patient care. ASCP advises TSH screening when patients are considered at-risk or demonstrate subtle or direct signs of thyroid dysfunction upon physical evaluation (ASCP, 2020).

Endocrine Society

The Endocrine Society recommends against testing for total or free T3 when evaluating LT4 dose in hypothyroid patients. They also recommend against ordering routine ultrasounds for patients without palpable abnormalities of the thyroid (Endocrine Society, 2018a, 2018b; Society, 2018). While routine thyroid ultrasounds should not be ordered without palpable abnormalities, thyroid vascularity assessments may be performed by color flow Doppler in patients who show overt hyperthyroidism evidenced by elevated free T4 and T3 and lower TSH values; color flow Doppler (a noninvasive ultrasound

test) may help diagnose Graves' hyperthyroidism and toxic nodular goiter from destructive thyroiditis (Endocrine Society, 2022).

European Thyroid Association (ETA)

Management of Thyroid Dysfunction following Immune Reconstitution Therapy (IRT) (Kahaly et al., 2018; Muller et al., 2019)

This guideline discusses IRT in the context of three clinical situations; "alemtuzumab (Lemtrada) treatment for active relapsing remitting multiple sclerosis (MS); (2) after treatment of human immunodeficiency virus (HIV) infected patients with highly active antiretroviral therapy (HAART); (3) following allogeneic bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT)"

The ETA recommends measuring TSH in all subjects before IRT. If TSH is abnormal, FT4 and FT3 are recommended to be measured.

Routine measurement of TPOAb or TRAb is not recommended before IRT.

TSH measurement is recommended post-IRT, and FT4 may also be routinely measured. If TSH is low (0.10–0.39 mU/L), another test is recommended within 1 month. If TSH is elevated, a repeat TSH test is recommended, along with FT4. If TSH is "suppressed" (<0.10 mU/L), TSH, FT4, and FT3 are recommended to be tested.

Following alemtuzumab, the ETA recommends "biochemical follow-up" with TSH testing every 3 months. Routine TSH monitoring is not recommended following HAART treatment in HIV patients, although TSH measurement should be performed if thyroid dysfunction is suspected.

Routine measurement of thyroid autoantibodies is not recommended in euthyroid patients during surveillance.

The ETA recommends "routine 3 monthly measuring of thyroid function to be continued for 4 years following the last alemtuzumab treatment" (Muller et al., 2019).

Thyroid Disorders Prior to and during Assisted Reproduction

The ETA recommends women of subfertile couples ("subfertile" is defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse) should be screened routinely for the presence of thyroid disorders. The ETA notes that severe thyroid dysfunction is linked to menstrual disorders as well as subfertility. In a meta-analyses that included mostly women with TSH levels above 4.0 mIU/L, treatment with levothyroxine was effective at increasing live birth rates (Poppe et al., 2021)

Management of Graves' Hyperthyroidism

The ETA notes measurement of TSH-R-stimulating antibody (TSH-R-Ab) as a "sensitive and specific" tool for rapid and accurate differential diagnosis for Graves' hypothyroidism. Differentiation of TSH-R-Ab is also "helpful and predictive" in Graves' patients during pregnancy/postpartum, as well as extrathyroidal manifestations.

The ETA also remarks that measurement of TSH-R-Ab levels prior to stopping antithyroid drug treatment (ATD).

For pregnant patients, maternal FT4 and TSH should be measured every 2 weeks after initiation of therapy, every 4 weeks after achieving the target value. All patients with history of autoimmune thyroid disease should have their TSH-R-Ab levels tested at first presentation with pregnancy, and if maternal TSH-R-Ab remains high (>3 times normal cutoff), monitoring the fetus for thyroid dysfunction throughout pregnancy is recommended (Kahaly et al., 2018).

In 2022, the ETA published guidelines for the management of GD in pediatric patients. Hyperthyroidism caused by GD is relatively rare in children and treatment options for pediatric patients are the same as those available to adults (ATD, radioactive iodine (RAI), or thyroid surgery). However, the risks and benefits of each modality are different in pediatric patients than they are in adult patients. The ETA recommends that "clinicians should be alert that GD may present with behavioral changes or declining academic performance in children. Measurement of serum TSH receptor antibodies is recommended for all pediatric patients with hyperthyroidism. Management recommendations include the first-line use of a prolonged course of methimazole/carbimazole ATD treatment (3 years or more), a preference for dose titration instead of block and replace ATD, and to avoid propylthiouracil use. Where definitive treatment is required either total thyroidectomy or RAI is recommended, aiming for complete thyroid ablation with a personalized RAI activity. We recommend avoiding RAI in children under 10 years of age but favor surgery in patients with large goiter. Pediatric endocrinologists should be involved in all cases" (Mooij et al., 2022).

Diagnosis and Management of Central Hyperthyroidism

The ETA also published a guideline regarding central hypothyroidism (CeH). Below are the relevant recommendations:

- "We recommend that the diagnosis of CeH should be considered in every subject with low serum concentrations of FT4 and low or normal TSH on a screening examination.
- We recommend that the diagnosis of CeH should be considered in neonates and children with clinical manifestations of congenital hypothyroidism but low or normal neonatal TSH screening.
- We suggest that the diagnosis of CeH should be considered in patients with a low serum concentration of FT4 and slight TSH elevations (< 10 mU/L, or inappropriately lower than expected on the basis of the hypothyroid state).
- We recommend screening for CeH all children with a familial history of CeH and/or failure to thrive, developmental delay, GH deficiency, delayed or precocious puberty, or other hypothalamic-pituitary defects or lesions.
- We recommend that CeH due to *IGSF1* defect should be ruled out in adolescents or adult patients with macroorchidism.
- We recommend screening for CeH all patients with a personal or familial history of hypothalamic-pituitary lesions or diseases, moderate to severe head trauma, stroke, previous cranial irradiation, hemochromatosis or iron overload, in particular when hypothyroid manifestations are present.
- We recommend screening for CeH all patients with hypothyroid manifestations associated with clinical findings pointing to a hypothalamic-pituitary disease (e.g., hyperprolactinemia, acromegalic features, diabetes insipidus, recurrent headaches, visual field defects), newborns with hypotonia

and/or prolonged jaundice, and/or signs of congenital hypopituitarism (e.g., micropenis with undescended testes), as well as children with developmental delay.

- We recommend that the onset of CeH should be evaluated in patients with hypothalamic/pituitary disease after the start of treatment with rhGH or estrogen.
- We recommend that the onset of CeH should be evaluated in patients on treatments with ligands of the retinoid X receptor (RXR), ipilimumab (or other checkpoint inhibitors), or mitotane."

Regarding diagnosis of CeH, the guideline recommends the following:

- "We recommend the combined determination of serum FT4 and TSH in order to evaluate the presence of CeH.
- We recommend that CeH diagnosis should be confirmed by the combined findings of serum FT4 concentrations below the lower limit of the normal range and inappropriately low/normal TSH concentrations on at least two separate determinations, and after exclusion of the conditions reported in Table 3.
- The isolated finding of low FT3 or total T3 concentrations is not indicative of CeH, but rather of nonthyroidal illness or deiodination defects (e.g., *SBP2* gene defect).
- In patients under follow-up for hypothalamic-pituitary disease, FT4 and TSH should be monitored during childhood at least biannually and later on a yearly basis, and we suggest that CeH diagnosis should be considered when serum FT4 falls in the lower quartile of the normal range, in particular when a FT4 decrease > 20% of previous values is seen (provided that the variables are measured by the same assay) despite a low or normal TSH.
- We suggest that the diagnosis of mild CeH (borderline low FT4, with inappropriately low TSH) should be supported by a combination of several other findings summarized in Table 4 (the relative application and importance of these tests and findings may vary in different settings)."

National Institute for Health and Care Excellence (NICE)

Thyroid Disease: Assessment and Management

NICE states to "consider" thyroid dysfunction tests for adults, children, and "young people" for the following indications:

- "a clinical suspicion of thyroid disease"
- New-onset atrial fibrillation
- Type 1 diabetes or other autoimmune disease
- Depression or unexplained anxiety
- For children and young people, consider tests for abnormal growths or unexplained change in behavior or school performance

NICE states not to test for thyroid dysfunction if a patient only has type 2 diabetes or if the patient has an unrelated acute illness.

Thyroid Disease Testing, continued

If secondary thyroid disease (pituitary disease) is not suspected, NICE states to "consider" measuring TSH. If TSH is "above reference range", measure FT4 in same sample; if TSH is "below reference range", measure FT4 and FT3 in same sample.

Measurement of both TSH and FT4 is to be considered for children or young people or if secondary thyroid dysfunction is suspected in adults. If TSH is below the reference range, FT3 should be measured. If symptoms in the above situations worsen, repeat the algorithms.

For adults with TSH levels above the reference range, TPO-Ab measurement may be considered. However, this testing should not be repeated. This applies to primary and subclinical hypothyroidism.

For children and young people, this measurement should be repeated when they become adults.

"For adults who are taking levothyroxine for primary hypothyroidism, consider measuring TSH every 3 months until the level has stabilised (2 similar measurements within the reference range 3 months apart), and then once a year." For adults with hypothyroidism symptoms after starting levothyroxine, consider measuring FT4 along with TSH.

For children ages 2 and over and young people taking levothyroxine for primary hypothyroidism, consider measuring FT4 and TSH at the following intervals:

"every 6 to 12 weeks until the TSH level has stabilised (2 similar measurements within the reference range 3 months apart), then every 4 to 6 months until after puberty, then once a year."

For children under 2, consider measuring FT4 and TSH at the following intervals:

"every 4 to 8 weeks until the TSH level has stabilised (2 similar measurements within the reference range 2 months apart), then every 2 to 3 months during the first year of life, and every 3 to 4 months during the second year of life."

For adults with untreated subclinical hypothyroidism or adults that have stopped treatment, consider measuring TSH and FT4 once a year if they are symptomatic, or once every 2-3 years if they are asymptomatic.

NICE states to consider measuring FT4 and TSH for children 2 and over with untreated subclinical hypothyroidism and TSH <10 mIU/liter at the following intervals: "every 3 to 6 months if they have features suggesting underlying thyroid disease, such as thyroid dysgenesis (an underdeveloped thyroid gland) or raised levels of thyroid autoantibodies, or every 6 to 12 months if they have no features suggesting underlying thyroid disease."

"Every 1-3 months for children ages 28 days-2 years with untreated subclinical hypothyroidism". TSH measurements may be stopped in children and young people if TSH has stabilized (defined as "2 similar measurements within the reference range 3 to 6 months apart") and there are no underlying features suggesting thyroid disease.

Differentiating between thyrotoxicosis with hyperthyroidism and thyrotoxicosis without hyperthyroidism may be performed by measuring TSH receptor antibodies (TRAbs). In children and young people, measuring TPO-Abs and TRAbs may be done to differentiate.

After radioactive iodine treatment, consider measuring FT3, FT4, and TSH every 6 weeks for the first 6 months, until TSH is within reference range.

"For adults, children and young people with TSH in the reference range 6 months after radioactive iodine treatment, consider measuring TSH (with cascading) at 9 months and 12 months after treatment."

"For adults, children and young people with TSH in the reference range 12 months after radioactive iodine treatment, consider measuring TSH (with cascading) every 6 months unless they develop hypothyroidism"

For patients taking antithyroid drugs for hyperthyroidism, consider measuring TSH, FT4, and FT3 every 6 weeks until TSH is within reference range, then TSH (with cascading) every 3 months until antithyroid drugs are stopped.

"For adults who have stopped antithyroid drugs, consider measuring: TSH (with cascading) within 8 weeks of stopping the drug, then TSH (with cascading) every 3 months for a year, then TSH (with cascading) once a year."

"For children and young people who have stopped antithyroid drugs, consider measuring: TSH, FT4 and FT3 within 8 weeks of stopping the drug, then TSH, FT4 and FT3 every 3 months for the first year, then TSH (with cascading) every 6 months for the second year, then TSH (with cascading) once a year."

"Consider measuring TSH every 6 months for adults with untreated subclinical hyperthyroidism. If the TSH level is outside the reference range, consider measuring FT4 and FT3 in the same sample."

"Consider measuring TSH, FT4 and FT3 every 3 months for children and young people with untreated subclinical hyperthyroidism."

"Consider stopping TSH measurement for adults, children and young people with untreated subclinical hyperthyroidism if the TSH level stabilises (2 similar measurements within the reference range 3 to 6 months apart)" (NICE, 2019).

Society for Maternal-Fetal Medicine (SMFM)

The SMFM recommends against screening asymptomatic pregnant women for subclinical hypothyroidism (SMFM, 2019). The SMFM states that "thyroid testing in pregnancy should be conducted for women "at risk," including known thyroid disease, symptoms of overt thyroid disease, suspected goiter, autoimmune medical disorders such as Type 1 diabetes mellitus", but also asserts that "routine treatment of subclinical hypothyroidism is not recommended" and "routine thyroid testing of women with hyperemesis gravidarum is not recommended".

Mitochondrial Medicine Society (MMS)

In 2017, the MMS created a working group to provide consensus-based recommendations for optimal management and care for patients with primary mitochondrial disease. From the guidelines, "initial triage stratification of critically ill mitochondrial patients should include a systemic assessment of all body systems since the disease is multisystemic and patients may develop new organ system involvement during an acute decompensation" and thyroid dysfunction can occur in patients with mitochondrial disease, as "both hypothyroidism and, to a far lesser extent, hyperthyroidism have been reported in patients with primary mitochondrial diseases". In addition to routine intensive-care management that might be undertaken for a critically ill patient, they recommend that "thyroid and adrenal function should be assessed in patients at times of critical illness and reassessed during a prolonged intensive care unit

stay. Hypo- and hyperglycemia can occur and regular blood glucose monitoring is needed." They also state that "an annual hemoglobin A1c (HgbA1c), thyroid-stimulating hormone, free thyroxine level (FT4), vitamin D, and screening for hypoparathyroidism (serum calcium, magnesium, phosphate, parathyroid hormone, vitamin D (25-OHD and 1,25-OHD); urine: creatinine, calcium, and phosphate) can be considered in individuals with mitochondrial diseases. In those with mtDNA deletions, which are more strongly associated with secondary endocrinopathies, annual screening is recommended" (Parikh et al., 2017).

V. Applicable State and Federal Regulations

A search of "thyroid" on the FDA website on December 31, 2020, yielded 106 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
80438	Thyrotropin releasing hormone (trh) stimulation panel; 1 hour This panel must include the following: Thyroid stimulating hormone (TSH) (84443x3)
80439	Thyrotropin releasing hormone (trh) stimulation panel; 2 hours This panel must include the following: Thyroid stimulating hormone (TSH) (84443x4)
83519	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, by radioimmunoassay (e.g., RIA)
84432	Thyroglobulin
84436	Thyroxine; total
84439	Thyroxine; free
84442	Thyroxine binding globulin (TBG)
84443	Thyroid stimulating hormone (TSH)
84445	Thyroid stimulating immune globulins (TSH)
84479	Thyroid hormone (T3 or T4) uptake or thyroid hormone binding ratio (THBR)
84480	Triiodothyronine T3; total (TT-3)
84481	Triiodothyronine T3; free
84482	Triiodothyronine T3; reverse

86376	Microsomal antibodies (e.g., thyroid or liver-kidney), each

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
4/26/22	Modified language in certain sections of criteria for clarity; added the term "Thyroid stimulating hormone (TSH)" to coverage criteria #1a to define the initialism; changed "primary and secondary" to "overt and subclinical" in coverage criteria #1b to align with updated clinical standards; and input appropriate timeframes for repeat TSH testing to be considered
2/20/23	Added coverage criterion #1dii: "d) For individuals capable of becoming pregnant who: i) Are undergoing evaluation for infertility. ii) ***Have experienced two or more pregnancy losses.***; also, added coverage criterion #1j: "For individuals diagnosed with primary mitochondrial disease, annual screening of TSH and fT4." And coverage criterion #1l: "For pediatric individuals with a clinical finding of failure-to-thrive."
9/27/23	The following changes were implemented: Coverage criteria #1 edited for clarity and consistency. "Policy Guidelines" section replaced with Note 1 (signs of hypothyroidism) and Note 2 (signs of hyperthyroidism), former Note 1 on testing in pregnancy becomes Note 3; coverage criteria #1.a.iv., frequency for hypothyroidism follow up changed from "6-12" to "every 6 weeks." Now reads: "iv) For individuals being treated for hypothyroidism, monitoring with TSH and fT4 testing every 6 weeks upon dosage change and annually in stable individuals."; coverage criteria #1b.v.a., frequency for hyperthyroidism follow up changed from "6-12" to "every 8 weeks." Now reads: "(a) In patients being treated for hyperthyroidism, repeat testing of TSH and fT4 should occur every 8 weeks."; former coverage criteria #1.e. pertaining to thyroid testing has been replaced with new

	coverage criteria #2 and coverage criteria #3: "2) For individuals who are pregnant or who are postpartum and who have symptoms of thyroid dysfunction (see Note 1 and Note 2), TSH and fT4 testing (once every 4 weeks) **MEETS COVERAGE CRITERIA** (see Note 3) and 3) For individuals who are pregnant or who are postpartum and who have been diagnosed with hyperthyroidism, total T4 (TT4), antithyroglobulin antibody (Tg-Ab), thyrotropin receptor antibodies (TRAb), and anti-thyroid peroxidase antibody (TPOAb) **MEETS COVERAGE CRITERIA** (see Note 3)."; thyroid antibody testing expanded beyond autoimmune thyroiditis, now allowing testing in hypothyroidism or hyperthyroidism, with testing restricted to once every 3 years. Former CC2, now CC4 reads: "4) For individuals with hypothyroidism or hyperthyroidism, testing for thyroid antibodies (once every three years) **MEETS COVERAGE CRITERIA**."; TBG added as not covered under any circumstances, former coverage criteria #4, now coverage criteria #6 now reads: "6) For the evaluation of the cause of hyperthyroidism or hypothyroidism, testing for thyrotropin-releasing hormone (TRH) or thyroxine-binding globulin (TBG) **DOES NOT MEET COVERAGE CRITERIA**."

Thyroid Disease Testing, continued

Urinary Tumor Markers for Bladder Cancer

Policy #: AHS – G2125	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/28/22, 10/17/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Bladder cancer is defined as a malignancy that develops from the tissues of the bladder. It is the most common cancer of the urinary system. The cancer typically arises from the urothelium, although it may originate in other locations such as the ureter or urethra (Lerner, 2020).

Tumor biomarkers are proteins detected in the blood, urine, or other body fluids that are produced by the tumor itself or in response to it. Urinary tumor markers may be used to help detect, diagnose, and manage some types of cancer including bladder cancer (Hottinger & Hormigo, 2011).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Urinary biomarkers (bladder tumor antigen (BTA) test, nuclear matrix protein (NMP22) test, or fluorescence in situ hybridization (FISH) UroVysion Bladder Cancer test) **MEET COVERAGE CRITERIA** in any of the following situations:

   a. As an adjunct in the diagnostic exclusion of bladder cancer for individuals who have an atypical or equivocal cytology

   b. As an adjunct in the monitoring of high-risk, non-muscle invasive bladder cancer

2. As an adjunct to cystoscopy or cytology in the monitoring of individuals with bladder cancer, the use of fluorescence immunocytology, (ImmunoCyt/uCyt) **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3. For the evaluation of hematuria, to screen for bladder cancer in asymptomatic individuals, to diagnose bladder cancer in symptomatic individuals, or for any other indication not discussed above, the following tests **DO NOT MEET COVERAGE CRITERIA**:

    a) Urinary biomarkers (bladder tumor antigen (BTA) test, nuclear matrix protein (NMP22) test, or fluorescence in situ hybridization (FISH) UroVysion Bladder Cancer test)

    b) Fluorescence immunocytology (ImmunoCyt/uCyt)

4. Any other urinary tumor markers for bladder cancer not mentioned above **DO NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Each year in the United States, about 75,000 individuals get bladder cancer, and about 16,700 individuals die from the disease (CDC, 2022). The American Cancer Society estimates about 82,290 new cases of bladder cancer and about 16,710 deaths in the United States in 2023 (ACS, 2023). Bladder cancer is the sixth most common cancer in the United States, affects men four times more frequently than women, and is typically diagnosed in individuals above the age of 40, with 73 the median age at diagnosis (DeGeorge et al., 2017; NCCN, 2019). Bladder cancer risk factors include smoking, a family history of the disease, pelvic radiation, obesity, diabetes, and chronic infection of the urinary tract.

Bladder cancer commonly presents as painless hematuria (blood in urine) and may be gross (visible) or microscopic. Gross hematuria tends to increase the likelihood of bladder cancer, but hematuria as a whole may be transient or due to non-cancer related causes (Perazalla, 2021). Other common symptoms of bladder cancer include pain or irritative and obstructive voiding symptoms such as urge incontinence, dysuria, straining, or nocturia. These symptoms are often mistaken for another condition such as kidney stones, can be temporary, and are not necessarily specific for bladder cancer (Lotan, 2022). In fact, hematuria is the most common symptom of bladder cancer, but a study reported a 13% prevalence rate of bladder cancer out of 6728 patients with hematuria (DeGeorge et al., 2017; Sutton et al., 2018). Approximately 70%-75% of patients present with superficial tumors (50 – 70% of which can recur but are usually not life threatening), and 25%-30% present as invasive tumors with a high risk of metastasis (Chou & Dana, 2010; Kaufman et al., 2009).

Cystoscopy (white light) is the gold standard for a diagnosis of bladder cancer. This procedure involves a bladder examination and urine sample for cytology. Any lesions are observed and recorded. Cystoscopy does not detect all malignancies or visualize the upper urinary tract. Furthermore, although cystoscopy is minimally invasive, it may be uncomfortable and promote anxiety, which can lead to suboptimal compliance with management recommendations. Fluorescent cystoscopy is somewhat more efficient at detecting tumors than white light cystoscopy; although, it comes with its own set of issues such as

higher false-positive rates and costs (Lotan, 2022; Mitra et al., 2012). Cytology, or the analysis of cells in urine, is often completed in addition to cystoscopy analysis.

Although cystoscopy has long been the gold standard for a diagnosis of bladder cancer, its high cost and unpleasant burden has led to the search for a non-invasive test that can match the high specificities and sensitivities set by cystoscopy. Urinary biomarkers including "Cell-free proteins and peptides, exosomes, cell-free DNA, methylated DNA and DNA mutations, circulating tumor cells, miRNA, lncRNA, rtRNA and mRNAs" have now been identified for bladder cancer diagnostic purposes (Lopez-Beltran et al., 2019). Urine is exposed to urothelial tissue in many different locations, and therefore has the potential to contain several biomarkers associated with cancer. Validation of these biomarkers could lessen the use of cystoscopy as well as increase the overall sensitivity for bladder cancer identification (D'Costa et al., 2016). However, because of the lower disease prevalence in a screening population, even in those at increased risk, the use of biomarkers for screening is not cost effective or recommended (Y. Lotan et al., 2009). Despite the promise of urine biomarkers, cystoscopy remains the procedure of choice both for initial diagnosis and for surveillance in previously treated patients.

Epigenetic changes may also play an important role in bladder cancer tumorigenesis. These changes are becoming more prevalent as identification rates increase due to improvements in high-throughput DNA sequencing technologies. Epigenetic changes can "regulate [the] gene expression outcome without changing the underlying DNA sequence" with alterations based on DNA methylation, nucleosome positioning, microRNA regulation and histone medications (Li, Duymich, Weisenberger, & Liang, 2016). All of these epigenetic-based changes are distorted in each human cancer type. "A substantial portion (76%) of all primary bladder tumors displays mutations in at least one chromatin regulatory gene. These mutations cause epigenetic dysregulation in bladder cancers (Li et al., 2016)."

Numerous other urinary biomarkers have been proposed as contributors to management of bladder cancer.

Other nuclear matrix proteins aside from NMP22 have been investigated. NMP52, BLCA-4, and BLCA-1 have all been studied as potential markers. Initial data for these markers appears promising, but most likely requires further evaluation (Mitra et al., 2019).

Cytokeratins, protein components of the cell structure, have also been identified as possible markers. Cytokeratins ("CK"), -8, -18, -19, and -20 have been considered for use in bladder cancer evaluation. However, further data is needed (Mitra et al., 2019).

Other markers that have been considered as potential indicators of bladder cancer include the following:

Telomerase is an enzyme that adds telomeres to the ends of chromosomes. This enzyme is only expressed in proliferating cells such as cancer cells, thereby lending credence to its use as a cancer marker. Despite its high sensitivity, its clinical application is limited, as the current assay used to detect telomerase is "significantly" affected by sample collection and processing (Mitra et al., 2019).

Hyaluronic acid is a polysaccharide that promotes tumor progression and metastasis. It is cleaved by *hyaluronidase*, which creates smaller fragments of the polysaccharide that further promote tumor angiogenesis. This pair of markers has been found to detect low-grade and low-stage disease with higher sensitivities than other markers, but requires further data for evaluation (Mitra et al., 2019).

Fibrin degradation products may also be useful in detection of cancer. High levels of vascular endothelial growth factor can increase the permeability of surrounding cellular structures, which cause serum proteins to "leak". These proteins are eventually degraded to fibrin, and then to fibrin degradation products (Mitra et al., 2022).

Survivin is an apoptosis inhibitor. Survivin is frequently elevated in cancers, but virtually undetectable in normal tissues. However, no commercial assays for survivin exist as of time of writing (Mitra et al., 2022).

Finally, *miRNA* markers have been considered for use in bladder cancer management. These markers are small sequences of non-coding RNA that contribute to gene expression regulation. MiRNAs-126, -200c, -143, and -222 have all been considered to have "promising" results (Mitra et al., 2022).

Proprietary Testing

The two most studied urinary biomarkers are bladder tumor antigen (BTA) and nuclear matrix protein 22 (NMP22). The BTA test is designed to detect complement factor H-related protein (hCFHrp) which is elevated in cancer cells. This test is available in both a quantitative and qualitive version, and its manufacturer-recommended cut-off is 14U/mL (Mahnert et al., 1999; Mitra, Birkman, & Penson, 2017). Similarly, the NMP22 test is designed to detect a protein that is more highly available in cancer cells than normal cells. In this case, cancer cells release more NMP22 into the urine following apoptosis than normal cells do. The NMP22 tests are also available in a quantitative and qualitative version, and its FDA-approved cut-off is 10U/mL (Grossman, Messing, Soloway, & et al., 2005; Mitra et al., 2017; Mitra et al., 2019; Zuiverloon, de Jong, & Theodorescu, 2017). A number of proprietary tests exist revolving around one of these two biomarkers; these tests include Abbott's "Alere NMP22 BladderCheck" and Quest's Bladder Tumor Antigen DetectR (Abbott, 2020; Quest, 2020)

The FDA has approved two additional tests for urinary biomarkers. One is *UroVysion*, which is designed to detect chromosomal alterations that are distinctive of bladder cancer. This test is a fluorescent in situ hybridization (FISH) assay that uses DNA probes to detect alterations (such as aneuploidies) on chromosomes 3, 7, and 17 or loss of the 9p21 locus. The second test is known as *ImmunoCyt* (or uCyt+) that uses a similar fluorescent technique to detect certain glycoproteins that are expressed solely on cancerous cells (Mitra et al., 2017; Mitra et al., 2019).

Recently, Pangea Laboratory has created a laboratory developed test termed Bladder *CARE*™ which measures the methylation status of specific DNA biomarkers in urine for the detection of bladder cancer via an at-home collection kit. This non-invasive test has not been approved by the FDA, is purported to be more cost-effective, and uses an epigenetic-based detection approach. Specifically, the methylation of bladder cancer DNA biomarkers are measured (Pangea, 2019a). As little as 5 ng of urine DNA from a 100 mL urine sample is required, and it has a limit detection of 0.1% leading to the identification of a single cancerous cell in a sample of 1,000 normal cells (Pangea, 2019a). The authors claim that Bladder *CARE*™ has a sensitivity of 94% and specificity of 86%, allowing for the identification of 88% of low-grade bladder cancer cases; these results are based on a study completed by Pangea Laboratory and Zymo Research which analyzes urine samples from 182 patients (97 with bladder cancer and 85 healthy controls) (Pangea, 2019b).

Another test, termed the Bladder EpiCheck test, has been developed by the Israeli company Nucleix. This non-invasive epigenetic urine test helps to detect bladder cancer with a panel of 15 DNA methylation biomarkers. Nucleix reports a sensitivity of 92%, a specificity of 88% and a negative

predictive value of 99% for the Bladder EpiCheck test; these results are based on a multi-center clinical study with 353 bladder cancer patients (Nucleix, 2015). Similar results have been reported by D'Andrea et al. (2019). However, this test is not available in the United States (Nucleix, 2015).

Another test, termed "UBC® Rapid" has been developed by the Swedish company ODL Biotech. This point-of-care test measures soluble fragments of cytokeratins 8 and 18 in urine samples. The test can produce results within 10 minutes and may be tested with hematuria-containing samples (IDL_Biotech, 2020). Ecke et al. performed a validation of this test, which encompassed 242 patients with bladder cancer (134 non-muscle-invasive low-grade tumors, 48 non-muscle-invasive high-grade tumors, 60 muscle-invasive high-grade tumors), 62 patients with non-evidence of disease [NED], and 226 healthy controls. The authors found a sensitivity of 38.8% for non-muscle-invasive low-grade bladder cancer, 75% for non-muscle-invasive high-grade bladder cancer and 68.3% for muscle-invasive high-grade bladder cancer. Specificity over the entire cohort was 93.8% (Ecke et al., 2018).

The URO17 assay by Protean Biodiagnostics, an immunohistochemistry-based test that detects the presence of the oncoprotein keratin 17 in bladder cancer and urogenital cancer. Unlike other urine-based test URO17 can detect patients with visible or invisible hematuria, which allows for early diagnosis. URO17 can also detect recurrent bladder cancer in patients under surveillance for relapse (NICE, 2021). The test has 100% sensitivity and 96% specificity for detecting bladder cancer from urine samples (Protean_Biodiagnostics, 2021).

Nonagen Bioscience released Oncuria, an in-vitro multiplex immunoassay, which detects protein biomarkers associated with bladder cancer in the urine. This non-invasive test detects ten proteins from a single urine sample in patients with hematuria with suspicion of bladder cancer. Biomarker levels are combined in a weighted algorithm to aid in the prediction of responding to Bacillus Calmette-Guerin (BCG) therapy in patients with intermediate to high-risk, early-stage bladder cancer (Nonagen_Bioscience, 2021).

Analytical Validity

Recently, Piao et al. (2019) have developed a way to differentiate patients with bladder cancer from patients with a nonmalignant hematuria without bladder cancer by measuring urinary cell-free microRNA expression. This study shows that the non-invasive measurement of urinary microRNA-6124 and microRNA-4511 can be used as a diagnostic tool with a sensitivity of >90% (Piao et al., 2019). This testing method will help to reduce the number of unnecessary cystoscopies in patients with hematuria that are being evaluated for bladder cancer.

The performance of an epigenetic-based bladder cancer detection tool has been evaluated by Fantony et al. (2017); the urine-based TWIST1/NID2 methylation assay has been analyzed for the detection of urothelial carcinoma via the addition of urine cytology. This multi-institutional study analyzed data from 172 patients. The authors note that "The AUC [area under the curve] for cytology alone with equivocal cytologies positive was 0.704, and improved to 0.773 with the addition of the DNA methylation assay ($p < 0.001$) (Fantony et al., 2017)." The authors conclude by stating that this TWIST1/NID2 methylation assay is a sensitive diagnostic tool that adds value to urine cytology for the detection of urothelial carcinoma, which is the most common type of bladder cancer.

Soubra and Risk (2015) found the sensitivity of fluorescent cystoscopy to be 0.92 and the sensitivity of white light cystoscopy to be 0.71; the specificity of fluorescent cystoscopy was lower at 0.57, and the specificity of white light cystoscopy was identified at 0.72. Furthermore, fluorescent cystoscopy's

sensitivity for carcinoma *in situ* (which is difficult to visualize) was measured at 0.924, while white light cystoscopy's sensitivity for carcinoma *in situ* was much lower at 0.605, but these differences tended to decrease on higher grade lesions (Soubra & Risk, 2015). Cytology is also a common analytic technique in addition to cystoscopy. Its overall sensitivity is low at 0.34 and its sensitivity for grade 1 and 2 tumors is even lower at 0.12 and 0.26, respectively (Yair Lotan & Roehrborn, 2003).

Breen et al. (2015) compared the sensitivity and specificity values of four diagnostic tests (cytology, NMP22, UroVysion, and CxBladder); CxBladder was found to have the highest sensitivity at 74% and cytology was identified with the highest specificity at 95%. The authors report comparable sensitivity values for cytology, NMP22, and UroVysion at 46%, 45.9% and 47.7% respectively (Breen et al., 2015). It is important to note that even though CxBladder is reported to have the highest sensitivity, the specificity (81.7%) is the lowest; the other tests were reported to have superior specificities with NMP22 at 88%, and UroVysion at 87.7% (Breen et al., 2015).

Sathianathen et al. (2018) published a study focusing on biomarkers in patients presenting with hematuria. This study encompassed BTA, NMP22, FISH, and uCyt+, as well as a fifth biomarker known as AssureMDx. Sensitivities ranged from 0.67 (BTA) to 0.95 (AssureMDx, second highest was uCyt+ at 0.83) while specificities ranged from 0.68 (BTA) to 0.93 (quantitative NMP22). However, this data is consistent with the previously published meta-analysis that covered all settings, not just hematuria (Chou et al., 2015). Cytology was also found to have superior specificity to all studied biomarkers; although, biomarkers tended to have better sensitivity. The authors concluded that, due to the high heterogeneity and small sample size, more studies were needed to validate biomarkers to replace diagnostic evaluation of hematuria (Sathianathen et al., 2018).

Although many studies emphasize the high validity of biomarkers such as NMP22 and BTA, these studies often have a large proportion of high-grade tumors which inflate the specificity and sensitivity; hence, the problem of identifying low-grade cancers remains. There may be changes at the genetic level in a low-grade cancer, but the proteins tested in the urine may still be relatively normal (D'Costa et al., 2016). Another issue is the conflicting results for the validity of the biomarkers. For example, the sensitivity of the quantitative NMP22 test has been found to range from as low as 0.26 to 1.00 with its specificity ranging from 0.49 to 0.98. Similarly, the *BTA STAT* test's sensitivity and specificity have been found to range from 0.29 to 0.91 and from 0.54 to 0.86 respectively (Zuiverloon et al., 2017). For comparison, a study found the sensitivity and specificity of flexible cystoscopy (out of 778 hematuria patients) to be 0.98 and 0.938, respectively (Sutton et al., 2018).

Dudley et al. (2019) have developed a novel high-throughput sequencing method that uses urine derived tumor DNA (utDNA) known as utDNA CAPP-Seq (Ucapp-Seq) to detect bladder cancer. This technique was used to analyze samples from 118 patients with early stage bladder cancer and 67 healthy adults. "We detected utDNA pretreatment in 93% of cases using a tumor mutation-informed approach and in 84% when blinded to tumor mutation status, with 96% to 100% specificity (Dudley et al., 2019)." These results show that utDNA can be used to diagnose early-stage bladder cancer with high sensitivity and specificity.

Hirasawa et al. (2021) studied the diagnostic performance of Oncuria™, a multiplex immunoassay urinalysis test for bladder cancer. Urine samples from 362 subjects with suspicion of bladder cancer were measured using Oncuria™ for ten biomarkers (A1AT, APOE, ANG, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA). Results of the test were confirmed by cystoscopy and tissue biopsy. "The Oncuria™ test achieved a strong overall diagnostic performance, achieving an overall AUC of 0.95, sensitivity and specificity values of 93% and 93%, respectively, and a negative predictive value (NPV) and positive

predictive value (PPV) of 99% and 65%, respectively. The Oncuria™ test shows promise for clinical application in the non-invasive diagnosis and surveillance bladder cancer, and potentially for screening at-risk, asymptomatic individuals (Hirasawa et al., 2021)."

Clinical Utility and Validity

A meta-analysis of 57 studies detailed the accuracy of several biomarkers for the diagnosis and surveillance of bladder cancer. These included the six FDA-approved tests (quantitative and qualitive NMP22, quantitative and qualitative BTA, FISH, and uCyt+) as well as a laboratory developed test that does not require FDA approval termed CxBladder. Sensitivities ranged from 0.57 (qualitative NMP22) to 0.82 (CxBladder); however, the CxBladder cohort was only comprised of one study. The specificities ranged from 0.74 (quantitative BTA) to 0.88 (qualitative NMP22). Sensitivity increased as a tumor progressed (higher grade or stage) with low accuracy for lower stage or grade tumors. A cytologic evaluation performed with a biomarker assessment increased sensitivity as well but missed about 10% of cases. Ultimately, the authors concluded that urinary biomarkers reported many false-positive results and failed to identify a large percentage of patients with bladder cancer (Chou et al., 2015). The authors also noted that this was the first study which focused on the measurement of clinical outcomes based on urinary biomarkers.

The ideal marker will be "easier, better, faster, and cheaper" (Schmitz-Dräger et al., 2015). Overall, although there have been numerous promising studies for the clinical utility of these urinary biomarkers, the biomarkers do not yet measure up to the standards set by cystoscopy as the primary method of diagnosis. Most of the biomarkers are yet to be well-validated and the ones that are, such as NMP22 and BTA, fall short of cystoscopy's standards (D'Costa et al., 2016). Furthermore, because of the lower disease prevalence in a screening population, even in those at increased risk, the use of biomarkers for screening is not cost effective or recommended (Y. Lotan et al., 2009). Although the cost of tests is non-clinical, it is still a crucial issue; the *BTA* and *NMP22* tests are relatively inexpensive at $25 but ImmunoCyt costs around $80 and the CxBladder and UroVysion cost $325 and $800, respectively (Zuiverloon et al., 2017). For comparison, a cystoscopy cost around $210 in 2016, and a cystoscopy with a biopsy cost about $370 (Halpern, Chughtai, & Ghomrawi, 2017). These biomarkers to date have not been highly recommended within any clinical guidelines. Therefore, the authors concluded that biomarkers have not had significant effect on clinical decision-making (Schmitz-Dräger et al., 2015). The majority of studies performed on these biomarkers did not focus on their ability to predict the course of cancer (D'Costa et al., 2016) but some biomarkers may play a role in the diagnosis or surveillance of bladder cancer in the future (Schmitz-Dräger et al., 2015). Even this may be a difficult barrier to cross; Meleth et al. (2014) prepared an assessment for the Agency for Healthcare Research and Quality that stated "although UroVysion is marketed as a diagnostic rather than a prognostic test, limited evidence from two small studies (total n=168) supported associations between test result and prognosis for risk of recurrence (Meleth et al., 2014)." The authors went on to note that no studies that established clinical utility were found.

D'Andrea et al. (2019) analyzed 357 urine samples from patients at five different centers under surveillance for non-muscle-invasive bladder cancer to investigate the clinical utility of the Bladder EpiCheck™ non-invasive urine test. A specificity of 88% was identified with this test, a negative predictive value of 94.4% for the detection of any cancer, and a negative predictive value of 99.3% for the detection of high grade cancer; the use of the Bladder EpiCheck™ test helped to improve the cancer recurrence predictive value by a difference of 16-22% (D'Andrea et al., 2019). This high-performing diagnostic test may help in the surveillance of non-muscle-invasive bladder cancer.

Tan et al. (2018) completed a systematic review to identify the diagnostic sensitivity and specificity of urinary biomarkers for the diagnosis of bladder cancer. The authors report that multi-target biomarker panels were more accurate than single biomarker targets, and that both the sensitivity and specificity of urinary biomarkers were higher in primary diagnostic scenarios compared to patients under surveillance (Tan et al., 2018). The authors note that "few biomarkers achieve a high sensitivity and negative predictive value," with single biomarkers reporting a sensitivity of 2-94% and specificity of 46-100%, and multi-target biomarkers reporting a sensitivity of 24-100% and specificity of 48-100% (Tan et al., 2018).

Mossanen et al. (2019) performed a cost analysis to characterize the costs of managing non-muscle-invasive bladder cancer (NMIBC). The authors created a Markov model with four health states: no evidence of disease, recurrence, progression and cystectomy, and death. Patients were stratified into three risk categories of low, intermediate, and high. The authors found that "cumulative costs of care over a 5-year period were $52,125 for low-risk, $146,250 for intermediate-risk, and $366,143 for high-risk NMIBC". The authors identified that the primary driver of cost was "progression to muscle-invasive disease requiring definitive therapy", which was found to contribute 81% and 92% to overall cost for intermediate and high-risk disease, respectively. Progression of disease was found to contribute 71% to overall cost for low-risk disease. The authors concluded that although protracted surveillance cystoscopy does contribute to management cost, progression of disease was the dominant factor in increasing cost of care (Mossanen et al., 2019).

Vasdev et al. (2021) studied the role of URO17™ biomarker in the diagnosis of bladder or urothelial cancer in new hematuria patients. Urine samples from 71 subjects were stained using the URO17™ immunobiomarker and results were compared to the biopsy and histology. URO17™ was shown to have an overall sensitivity of 100%, specificity of 92.6%, positive predictive value of 0.957, and negative predictive value of 1. URO17™ investigation was positive in every case of urothelial malignancy. According to the authors, URO17™ test can help improve "diagnostic capabilities in primary care, reduce the number of referrals to Urology department, and reduce the number of unnecessary invasive procedures for new patients with a suspected urinary bladder cancer" (Vasdev et al., 2021).

V. Guidelines and Recommendations

National Comprehensive Cancer Network

The NCCN has stated that "Urine molecular tests for urothelial tumor markers are now available. Many of these tests have a better sensitivity for detecting bladder cancer than urinary cytology, but specificity is lower. Considering this, evaluation of urinary urothelial tumor markers may be considered during surveillance of high-risk non-muscle-invasive bladder cancer. However, it remains unclear whether these tests offer additional information that is useful for detection and management of non-muscle-invasive bladder tumors. Therefore, the panel considers this to be a category 2B recommendation (NCCN, 2018, 2019, 2020a)."

The NCCN previously stated that an FDA-approved urinary biomarker test such as fluorescence in situ hybridization (FISH) or nuclear matrix protein 22 may be considered in monitoring for recurrence (NCCN, 2018). However, updated NCCN (2019, 2020a) guidelines no longer address these biomarker tests.

National Academy of Clinical Biochemistry (NACB) Laboratory Medicine

The NACB Laboratory Medicine Practice Guidelines do not recommend use of any FDA–approved urinary tumor marker tests for the diagnosis of bladder tumors or for monitoring bladder cancer patients. The guideline states that "There are no prospective clinical trial data that establish the utility of

any of the FDA cleared markers or the proposed markers for increasing survival time, decreasing the cost of treatment or improving the quality of life of bladder cancer patients (NACB, 2010)." The NACB is now known as the AACC, or American Association for Clinical Chemistry, and have not since released any further updates on this topic.

American Urological Association (AUA)

The AUA's guidelines on the diagnosis, evaluation and follow-up of asymptomatic microhematuria (AMH) in adults do not recommend use of urine markers (NMP22, BTA-stat, UroVysion) as part of routine evaluation (Davis et al., 2012).

The AUA and Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction (SUFU) published a guideline on microhematuria in 2020. In it, they remark that "Clinicians should not use urine cytology or urine-based tumor markers in the initial evaluation of patients with microhematuria", stating that "insufficient evidence exists that routine use would improve detection of bladder cancer." However, the guideline states that "Clinicians may obtain urine cytology for patients with persistent microhematuria after a negative workup who have irritative voiding symptoms or risk factors for carcinoma in situ." Overall, the guideline states that "the panel does not recommend using urine cytology or urine-based tumor markers in the initial evaluation of MH [microhematuria] because, to date, markers have not demonstrated incrementally additive information to cystoscopy in the MH population, not have they been found to be of sufficient predictive value to obviate cystoscopy" (Barocas et al., 2020)

The AUA and Society of Urologic Oncology (SUO) joint guidelines on Diagnosis and Treatment of Non-Muscle Invasive Bladder Cancer (NMIBC) do not recommend using urinary biomarkers to replace cystoscopy when monitoring NMIBC (grade B), although a clinician can use biomarkers to evaluate a patient's response to Bacillus Calmette-Guerin (BCG) therapy or a separate cytology such as FISH or ImmunoCyt. However, a urinary biomarker should not be used for monitoring a patient with a normal cystoscopy and a history of low-risk cancer (Chang et al., 2020). This 2016 guideline was amended in 2020, but no relevant changes were identified.

The 2021 American Urologic Association (AUA) annual meeting included a guideline amendment update for non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) to the 2020 guidelines. According to the update, a clinical should not use urinary biomarkers in place of cystoscopy. "In a patient with a history of low-risk cancer and a normal cystoscopy, a clinician should not routinely use a urinary biomarker or cytology during surveillance. In a patient with NMIBC, a clinician may use biomarkers to assess response to intravesical BCG (UroVysion® FISH) and adjudicate equivocal cytology (UroVysion® FISH and ImmunoCyt™)" (AUA/SUO, 2020). The panel does acknowledge the uptake of Cxbladder in clinical practice; however, there is a lack of high quality evidence in the potential replacement of cystoscopy with Cxbladder (AUA, 2021).

Similarly, the joint guidelines between the AUA, the SUO, the American Society of Clinical Oncology (ASCO), and the American Society for Radiation Oncology (ASTRO) regarding non-metastatic muscle-invasive bladder cancer note that molecular biomarkers may be important for staging cancer and deciding a course of treatment soon. Nevertheless, at this time the biomarkers have not been properly validated (Chang et al., 2017).

U.S. Preventive Services Task Force (USPSTF)

The USPSTF concluded in 2011 that there was insufficient evidence to evaluate screening for bladder cancer in asymptomatic adults, assigning a grade I to this recommendation. Since then, there have been no further guidelines published on this topic by the USPSTF (Moyer, 2011).

In 2021, the USPSTF published the following statement regarding bladder cancer screening in adults: "Literature scans conducted in November 2021 in the MEDLINE and PubMed databases and the Cochrane Library showed a lack of new evidence to support an updated systematic review on the topic at this time (USPSTF, 2021)."

3rd International Consultation on Urological Diseases & Société Internationale d'Urologie (ICUD-SIU)

With a level of evidence of 3 and a grade of "B", the ICUD-SIU recommends, "examination of urine cytology must be a part of the expectant management or active surveillance protocol." Concerning the surveillance strategies for NMIBC, "Surveillance strategies following a negative 3 months surveillance cystoscopy should be: (1) for low risk disease, cystoscopy 6–9 months later and annually thereafter; consider cessation following five recurrence-free years. No upper tract imaging necessary unless hematuria present; (2) for intermediate risk, cystoscopy with cytology every 3–6 months for 2 years; then every 6–12 months during years 3 and 4; then annually for lifetime. Upper tract imaging every 1–2 years; (3) for high risk, cystoscopy with cytology every 3 months for 2 years; then every 6 months during years 3 and 4; then annually for lifetime [Level of evidence: 3; Grade C] (Monteiro et al., 2018)."

National Cancer Institute (NCI)

In the 2022 update to the NCI's *Bladder and Other Urothelial Cancers Screening (PDQ®)—Health Professional Version*, the NCI states that "There is inadequate evidence to determine whether screening for bladder and other urothelial cancers has an impact on mortality… Based on fair evidence, screening for bladder and other urothelial cancers would result in unnecessary diagnostic procedures with attendant morbidity (NCI, 2022)."

European Association of Urology (EAU)

The EAU has published guidelines on non-muscle-invasive bladder cancer. Regarding urinary molecular marker tests, the EAU has stated that "Driven by the low sensitivity and low negative predictive value of urine cytology, numerous urinary tests have been developed. None of these markers have been accepted for diagnosis or follow-up in routine practice or clinical guidelines (Babjuk et al., 2017)." Further, as an exploratory measure after hematuria or after other bladder cancer symptoms have been identified, the EAU states that "It is generally accepted that none of the currently available tests can replace cystoscopy. However, urinary cytology or biomarkers can be used as an adjunct to cystoscopy to detect missed tumours, particularly CIS [carcinoma *in situ*]. In this setting, sensitivity for high-grade tumours and specificity are particularly important (Babjuk et al., 2017)." Finally, the EAU states that currently, there is no urinary marker with the ability to replace cystoscopy.

An update to these guidelines was published in 2020. In it, the EAU concluded that "Cystoscopy is necessary for the diagnosis of bladder cancer" and that "Urinary cytology has high sensitivity in high-grade tumours including carcinoma in situ." The EAU remarks that "There is no known urinary marker specific for the diagnosis of invasive BC [bladder cancer]" (Witjes et al., 2020).

An update to guidelines on non-muscle-invasive bladder cancer (NIBC) was published in 2022. The EAU concluded that urinary molecular marker tests cannot replace cystoscopy in routine practice, "but the knowledge of positive test results (microsatellite analysis) can improve the quality of follow-up

cystoscopy." Diagnosis ultimately depends on "cystoscopy examination of the bladder and histological evaluation of sampled tissue" (Babjuk et al., 2022).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On April 16, 1997, the FDA approved the *Bard BTA stat™ Test*, created by Bard Diagnostic Sciences Inc. From the FDA site: "the BTA stat test is an *in vitro* diagnostic immunoassay indicated for the qualitative detection of bladder tumor associated antigen in urine of persons diagnosed with bladder cancer. This test is indicated for use as an aid in the management of bladder cancer patients in conjunction with cystoscopy."

On April 15, 1998, the FDA approved the *BTA TRAK™ Test*, created by Bard Diagnostic Sciences Inc. From the FDA site: "the BTA TRAK test is an *in vitro* diagnostic immunoassay indicated for the quantitative detection of bladder tumor associated antigen in human urine. This test is indicated for use as an aid in the management of bladder cancer patients in conjunction with cystoscopy."

On July 2, 1996, the FDA approved the *MATRITECH NMP22™ TEST KIT*, created by Alere Scarborough Inc. From the FDA site: "The Matritech NMP22 Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of nuclear matrix protein NMP22 in stabilized voided urine."

On July 30, 2002, the FDA approved the *NMP22 BladderChek*, created by Matritech Inc. From the FDA site: "The Matritech NMP22 BladderChek Test is indicated for professional and prescription home use as an aid in monitoring bladder cancer patients, in conjunction with standard diagnostic procedures." This assay is qualitative.

On January 24, 2005, the FDA approved the *UROVYSION BLADDER CANCER KIT*. From the FDA site: "The UroVysion Bladder Cancer Kit (UroVysion Kit) is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer."

On February 23, 2000, the FDA approved the *ImmunoCyt,* created by Diagnocure Inc. From the FDA site: "ImmunoCyt is a qualitative direct immunofluorescence assay intended for use in conjunction with cytology to increase overall sensitivity for the detection of tumor cells exfoliated in the urine of patients previously diagnosed with bladder cancer. ImmunoCyt is indicated for use as an aid in the management of bladder cancer in conjunction with urinary cytology and cystoscopy (FDA, 2018)."

All of the FDA-approved tests apart from ImmunoCyt are approved for both diagnosis and surveillance of bladder cancer whereas ImmunoCyt is only approved for surveillance (Darwiche et al., 2015).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86294	Immunoassay for tumor antigen, qualitative or semiquantitative (e.g., bladder tumor antigen)
86316	Immunoassay for tumor antigen; other antigen, quantitative, each
86386	Nuclear matrix protein 22 (nmp22), qualitative
88120	Cytopathology, in situ hybridization (e.g., FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen, manual
88121	using computer-assisted technology (for morphometric in situ hybridization on cytologic specimens other than urinary tract, see 88367, 88368) (for more than 5 probes, use 88399)
88346	Immunofluorescence, per specimen; initial single antibody stain procedure
88350	Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
86294	Immunoassay for tumor antigen, qualitative or semiquantitative (e.g., bladder tumor antigen)
0365U	Oncology (bladder), analysis of 10 protein biomarkers (A1AT, ANG, APOE, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA) by immunoassays, urine, algorithm reported as a probability of bladder cancer
0366U	Oncology (bladder), analysis of 10 protein biomarkers (A1AT, ANG, APOE, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA) by immunoassays, urine, algorithm reported as a probability of recurrent bladder cancer
0367U	Oncology (bladder), analysis of 10 protein biomarkers (A1AT, ANG, APOE, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA) by immunoassays, urine, diagnostic algorithm reported as a risk score for probability of rapid recurrence of recurrent or persistent cancer following transurethral resection

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VII. Evidence-based Scientific References

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VIII. Revision History

Revision Date	Summary of Changes
4/28/22	Modified wording in certain coverage criteria to ensure clarity.
10/17/23	Modified wording in overall criteria, and modified formatting in coverage criteria #3 to ensure clarity.

Urine Culture Testing for Bacteria

Policy #: AHS – G2156	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/24/22 (see Section VIII)

3.

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Bacteriuria is the presence of bacteria in the urine. Urinary tract infections (UTIs) can occur in the urinary system and can be either symptomatic or asymptomatic. UTIs can include cystitis, an infection of the bladder or lower urinary tract; pyelonephritis, an infection of the upper urinary tract or kidney; urosepsis; urethritis; and male-specific conditions, such as bacterial prostatitis and epididymitis (Bonkat et al., 2023; Hooton & Gupta, 2023). Typically, in an infected person, bacteriuria and pyuria (the presence of pus in the urine) are present and can be present in both symptomatic and asymptomatic UTIs. A urine culture can be performed to determine the presence of bacteria and to characterize the bacterial infection (Meyrier, 2023).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request

1. For pregnant individuals, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) for any urinary tract infection (UTI) **MEETS COVERAGE CRITERIA**.

2. For asymptomatic individuals undergoing urological interventions which breach the mucosa, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) prior to the procedure **MEETS COVERAGE CRITERIA**.

3. For individuals exhibiting at least one sign or symptom of a possible UTI or bacteriuria (see Note 1 below), urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **MEETS COVERAGE CRITERIA**.

4. To assess pyelonephritis, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **MEETS COVERAGE CRITERIA**.

5. For all other instances of asymptomatic UTI or asymptomatic bacteriuria not described above, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **DOES NOT MEET COVERAGE CRITERIA**.

6. For individuals that show evidence of clinical resolution of infection, follow-up urine culture testing for an uncomplicated UTI **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7. Urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:

   b. As a part of initial screening for asymptomatic prostatitis.

   c. As a part of assessment or prognosis of prostate biopsy.

NOTE 1: Signs and symptoms of UTI/bacteriuria include (CDC, 2021)

- Fever
- Urgency to urinate
- Feeling the need to urinate despite having an empty bladder
- Increased frequency of urination
- Dysuria
- Suprapubic tenderness
- Pyuria
- Hematuria
- Cloudy urine
- Lower Back and Side (flank) pain
- Nausea
- Vomiting
- Chills
- Night sweats
- Pelvic pressure
- Change in urine smell

- Abnormal urinalysis findings

III. Scientific Background

Urinary tract infections (UTIs) can be either symptomatic or asymptomatic and can be classified as uncomplicated or complicated. Uncomplicated UTIs are "acute, sporadic or recurrent cystitis limited to non-pregnant women with no known relevant anatomical and functional abnormalities within the urinary tract or comorbidities" (Bonkat et al., 2023). All other UTIs that are not defined as uncomplicated are complicated UTIs. Meaning in a narrower sense UTIs in a patient with an increased chance of a complicated course: i.e. all men, pregnant women, patients with relevant anatomical or functional abnormalities of the urinary tract, indwelling urinary catheters, renal diseases, and/or with other concomitant immunocompromising diseases for example, diabetes (Bonkat et al., 2023)". *Escherichia coli* is the most common cause of complicated UTIs; however, "other uropathogens include other Enterobacteriaceae (such as *Klebsiella* spp and *Proteus* spp), *Pseudomonas*, enterococci, and staphylococci (methicillin-sensitive *Staphylococcus aureus* [MSSA] and methicillin-resistant *S. aureus* [MRSA])" (Hooton & Gupta, 2021). Even though both bacteriuria and pyuria are often present in UTIs, their presence alone is not indicative of a symptomatic infection.

The presence of bacteriuria does not guarantee negative outcomes for a patient. In fact, the paradigm of the sterility of the bladder environment has changed considerably over recent years. At least for females, the presence of female urinary microbiota (FUM) is believed to occur naturally and has been documented using sensitive bacterial DNA screening tests on asymptomatic females (Brubaker & Wolfe, 2016). Beneficial microbes, such as vaginal strains of *Lactobacillus*, can inhibit the grown of uropathogenic bacteria, including *E. coli* (Aroutcheva et al., 2001; Brubaker & Wolfe, 2016). Over-prescribing antibiotics, especially in cases of asymptomatic bacteriuria, can lead to both an eradication of beneficial bacterial flora and an emergence of antibiotic-resistant bacteria. Prescribing antibiotics as a prophylactic measure or in the instance of asymptomatic bacteriuria is detrimental because it is of limited value and can also increase incidences of drug-resistance. A study in 2002 by Harding and colleagues show that antibiotic treatment in diabetic women with asymptomatic bacteriuria did not result in a decrease of future symptomatic UTIs as compared to the control group; in fact, the experimental group had higher rates of adverse antimicrobial reactions (Harding, Zhanel, Nicolle, & Cheang, 2002). Even though the evidence-based guidelines by various societies, such as the EAU (Bonkat et al., 2023) and SHEA (SHEA, 2019), do not recommend performing urine testing or treatment for asymptomatic bacteriuria, inappropriate treatment is still occurring; in fact, one study by Cope and colleagues show that 32% of catheter-associated cases of asymptomatic bacteriuria and asymptomatic UTI received inappropriate treatment (Cope et al., 2009). The Antimicrobial Resistance Epidemiological Survey on Cystitis (ARESC) shows that up to 10.3% of *E. coli* in UTIs are "resistant to at least three different classes of antimicrobial agents" with ampicillin having the highest degree of resistance (48.3%). This is a large study of 4264 women from ten different countries to show that antibiotic-resistance is of international importance (Schito et al., 2009).

Analytical Validity

Urinalysis (UA) to detect nitrite and leukocyte esterase to indicate the presence of bacteria is an accepted laboratory practice. One report, though, has shown that the use of nitrite has "a sensitivity of

3%, a specificity of 97%, and a negative predictive value of 55% et al., 1992)". (Devillé et al., 2004). A 2004 meta-analysis study asserts that the "sensitivities of the combination of both tests vary between 68 and 88% in different patient groups, but positive test results have to be confirmed." They did note that the accuracy of the leukocyte esterase testing was higher in urology patients with a diagnostic odds ratio (DOR) of 276 as compared to the accuracy of nitrites (for example, in elderly patients DOR = 108).

Urine culture is considered a "gold standard" for detecting the presence of bacteria in urine (Graham & Galloway, 2001; Schmiemann et al. , 2010). That being said, "the interpretation of culture results can be considered as more of an art than a science. A urine culture result depends on so many variables, such as appropriate collection, transport, and the limits of the methods of detection. The reliability of single positive urine culture in diagnosing UTI is only 80%, rising to 90% if a repeat culture shows identical results (Graham & Galloway, 2001)." This is using the definition of bacteriuria as being 10^5 bacteria/ml of urine.

A potential future alternative to the urine culture could be multiplex PCR-based molecular testing, which Wojno et al. (2020) had found to be noninferior to urine culture for detection and identification of the bacteria. Agreement between the two testing methods was 90%, which exceeded the 85% noninferiority threshold. The multiplex PCR was also able to detect bacteria in 36% of symptomatic patients who had negative urine cultures and detected more polymicrobial infections than urine culture in a shorter amount of time (6 hours vs 48 hours for urine culture). (Wojno et al., 2020)

Clinical Utility and Validity

A study by Bruyere et al. (2010) using 353 patients undergoing prostate biopsy show that the routine use of obtaining a pre-operative urine culture is not clinically relevant to positive outcomes. "Of the 353 men, 12 had a pre-biopsy-positive bacterial culture and underwent prostate biopsy without any infections complication. Fifteen patients with a negative pre-biopsy culture developed a post-biopsy-positive bacterial culture, but remained asymptomatic without any treatment. Only four men from the group without pre-biopsy bacteriuria developed an infectious complication, requiring 3 weeks of antibiotic therapy." Both experimental and control groups had similar rates of complication, suggesting "that routine urine bacterial culture before prostate biopsy is not useful when antibiotic prophylaxis and enema are performed."

The method of obtaining the urine sample for culture testing is important. This is especially true for children. A 2017 study of 4808 acutely ill children demonstrated that there was modest agreement between the results obtained if the test was conducted by a research laboratory versus a health service laboratory; however, the method of obtaining the urine sample did have significance. The calculated areas under the receiver-operator curve (AUC) for UTI ranged from 0.75-0.86 if the sample was obtained using a clean-catch method versus AUC values of 0.65-0.79 if the sample was obtained using "nappy pad samples". The authors conclusions were that urine cultures did not necessarily have to be sent to a research lab for testing, but that "primary care clinicians should try to obtain clean catch samples, even in very young children" (Birnie et al., 2017). A smaller study of 83 infants compared the use of urine obtained either via bladder catheterization or suprapubic aspiration (SPA) (Eliacik et al., 2016). All 83 infants had previously tested positive using urine culture samples obtained via bladder catheterization. Then, they had samples removed by SPA. The SPA samples were used in both urinalysis and urine

culture testing, and "only 24 (28.9%) and 20 (24%) yielded positive urine culture and abnormal urinalysis data, respectively." This indicates a 71.1% false-positive result rate if the urine sample is obtained using bladder catheterization. "In infants younger than 12 months, SPA is the best method to avoid bacterial contamination, showing better results than transurethral catheterization (Eliacik et al., 2016)."

Another study (Ducharme et al., 2007) researched the use of either urine cultures and/or reagent test strips for use in diagnosing UTIs in elderly patients. The study consisted of 100 elderly patients with one group having no symptoms and non-infectious complaints and a second group "presenting with acute confusion, weakness or fever but no apparent urinary symptoms". Their results show that "of the 33 positive cultures, 10 had negative reagent strips. Thirteen of the 14 positive nitrite tests were culture positive for a specificity of 92.8% and a sensitivity of 36.1%. Positive cultures did not infer a diagnosis of UTI. Of the 67 positive reagent strips, 41 (61.2%) were associated with negative cultures." They conclude that, "in the elderly, reagent testing is an unreliable method of identifying patients with positive blood cultures. Moreover, positive urine culture rates are only slightly higher in patients with vague symptoms attributable to UTI than they are in (asymptomatic) patients treated for non-urologic problems, which suggests that many positive cultures in elderly patients with non-focal systemic symptoms are false-positive tests reflecting asymptomatic bacteriuria and not UTIs (Ducharme et al., 2007)."

A study by Price et al. (2016) show that using an enhanced quantitative urine culture (EQUC) increased the detection of microorganisms in UTIs. This study consisted of 150 female patients using an initial UTI symptom assessment questionnaire to divide them into symptomatic and asymptomatic groups. Both sets underwent culture testing using both conventional urine culture testing and an EQUC method. "Compared to expanded-spectrum EQUC, standard urine culture missed 67% of uropathogens overall and 50% in participants with severe urinary symptoms. Thirty-six percent of participants with missed uropathogens reported no symptom resolution after treatment by standard urine culture results." Their protocol resulted in an "84% uropathogen detection relative to 33% detection by standard urine culture" (Price et al., 2016).

Cantey et al. (2015) evaluated the utility of a Gram stain relative to UA. In reviewing 312 pediatric patients with suspected UTIs who had urine cultures, UA, and Gram stain performed, the researchers concluded that the UA "has excellent negative predictive value that is not enhanced by urine Gram stain and that antibiotic selection did not vary based on the urine Gram stain result." When compared to the urine Gram stain, the UA had equal sensitivity (97.3% vs 97.5%) and a higher specificity (85% vs 74%). This could allow the UA to take precedent as a test performed over the Gram stain due to its increased efficiency and lower cost.

Petty et al. (2019) evaluated the risk factors and clinical outcomes of treating asymptomatic bacteriuria (ASB) in hospitalized patients. 2733 patients with ASB (defined as "positive urine culture without any documented signs or symptoms attributable to urinary tract infection") were included. 2259 patients were treated with antibiotics for a mean of 7 days. Certain characteristics tended to correlate with ASB treatment, such as positive urinalysis (odds ratio [OR] = 2.83), leukocytosis (OR = 1.55), and dementia (OR = 1.57). However, treatment of ASB was found to be associated with longer duration of hospitalization after urine testing (4 vs 3 days; relative risk, 1.37), although no other differences in

secondary outcomes were identified. The authors concluded that "hospitalized patients with ASB commonly receive inappropriate antibiotic therapy. Antibiotic treatment did not appear to be associated with improved outcomes; rather, treatment may be associated with longer duration of hospitalization after urine testing." The authors also recommended stewardship efforts to reduce inappropriate treatment (Petty et al., 2019).

Coussement et al. (2019) investigated the prevalence of asymptomatic bacteriuria among kidney transplant patients beyond two months post-transplant. The authors identified 500 post-transplant patients, of which 17 had asymptomatic bacteriuria (3.4%). Further, of the 76 patients that were 2-12 months post-transplant, only 1 had asymptomatic bacteriuria, and of the other 424 patients, 16 patients had asymptomatic bacteriuria. The authors concluded that the prevalence of asymptomatic bacteriuria past the second month of kidney transplant was low and that further studies were needed to ascertain the cost-effectiveness of the screen-and-treat strategy in this population (Coussement et al., 2019). This finding regarding screening and treating AB was confirmed by Fontserè et al. (2021), who found that the "treatment of AB diminished the microbiological cure and increased the rates of microbiologic relapses and reinfections… treated AB patients showed a trend of developing symptomatic urinary tract infection in the following six months."

IV. Guidelines and Recommendations

Choosing Wisely

Choosing Wisely, an initiative by the American Board of Internal Medicine (ABIM) Foundation, consists of several national organizations representing medical specialists that write recommendations within their respective field to help choose care based on scientific evidence and to help reduce testing redundancy.

2019 AMDA-The Society for Post-Acute and Long-Term Care Medicine (AMDA)

In 2019, the AMDA updated their earlier 2017 Choosing Wisely guideline concerning the use of urine cultures. Due to overuse of antibiotics and overtreatment of UTIs, they state "Don't obtain urine tests until clinical criteria are met." Since the urine culture would have a high likelihood of yielding a positive result in an otherwise asymptomatic case, this "contributes to the over-use of antibiotic therapy in this setting, leading to an increased risk of diarrhea or other adverse drug events, resistant organisms and infection due to *Clostridioides difficile*." They also note that "the finding of asymptomatic bacteriuria may lead to an erroneous assumption that a UTI is the cause of an acute change of status, hence failing to detect or delaying the timelier detection of 5 signs and symptoms likely indicative of uncomplicated cystitis. These include dysuria, and one or more of the following: frequency, urgency, supra-pubic pain or gross hematuria". (AMDA, 2019).

2018 American Academy of Pediatrics-Section on Nephrology (ASPN) and the American Society of Pediatric Nephrology (AAP)

The AAP Section on Nephrology and the ASPN issued a joint Choosing Wisely recommendation stating, "Avoid ordering follow-up urine cultures after treatment for an uncomplicated urinary tract infection (UTI) in patients that show evidence of clinical resolution of infection. Studies have shown that clinical

resolution of infection is adequate for determining effectiveness of antibiotic therapy after treatment for a UTI" (AAP & ASPN, 2018).

2016 American Academy of Pediatrics (AAP)

The AAP updated their Choosing Wisely recommendation in 2016: "Avoid the use of surveillance cultures for the screening and treatment of asymptomatic bacteriuria." There is no evidence that surveillance urine cultures or treatment of asymptomatic bacteriuria is beneficial. Surveillance cultures are costly and produce both false positive and false negative results. Treatment of asymptomatic bacteriuria is harmful and increases exposure to antibiotics, which is a risk factor for subsequent infections with a resistant organism. This also results in the overall use of antibiotics in the community and may lead to unnecessary imaging" (AAP, 2016).

2019 Society for Healthcare Epidemiology of America (SHEA)

The SHEA recommendation in Choosing Wisely is more encompassing: "Don't perform cultures (e.g. urine, blood, sputum cultures) or test for *C. difficile* unless patients have signs or symptoms of infection. Tests can be falsely positive leading to over diagnosis and overtreatment. Although important for diagnosing disease when used in patients with appropriate signs or symptoms, these tests often are positive when an infection is not present. For example, in the absence of signs or symptoms, a positive blood culture may represent contamination, a positive urine culture could represent asymptomatic bacteriuria, and a positive test for *C. difficile* could reflect colonization. There are no perfect tests for these or most infections. If these tests are used in patients with low likelihood of infection, they will result in more false positive tests than true positive results, which will lead to treating patients without infection and exposing them to risks of antibiotics without benefits of treating an infection" (SHEA, 2019).

European Association of Urology (EAU)

The EAU has 2020 guidelines for urological infections. In 2022, the guidelines were updated with minor changes. With respect to **asymptomatic bacteriuria**, they state (all with a 'Strong' strength of rating), "Do not screen or treat asymptomatic bacteriuria in the following conditions:

- Women without risk factors;
- Patients with well-regulated diabetes mellitus;
- Post-menopausal women;
- Elderly institutionalised patients;
- Patients with dysfunctional and/or reconstructed lower urinary tracts;
- Patients with renal transplants;
- Patients prior to arthroplasty surgeries;
- Patients with recurrent urinary tract infections."

They do recommend with a 'Strong' rating to "screen for and treat asymptomatic bacteriuria prior to urological procedures breaching the mucosa" and a 'Weak' rating to "screen for and treat asymptomatic

bacteriuria in pregnant women with standard short course treatment." They do recommend to "diagnose **recurrent UTI** by urine culture" with a 'Strong' rating. Please note that recurrent UTI indicates that the occurrences are symptomatic. It is further specified that "A urine culture must therefore be taken prior to such interventions".

With respect to **uncomplicated cystitis**, they give a 'Strong' rating to only perform urine culture analysis "in the following situations:

- Suspected acute pyelonephritis;
- Symptoms that do not resolve or recur within four weeks after the completion of treatment;
- Women who present with atypical symptoms;
- Pregnant women."

The EAU gives a 'Weak' recommendation to "use urine dipstick testing for diagnosis of acute uncomplicated cystitis."

In cases of uncomplicated **pyelonephritis**, the EAU recommends with a 'Strong' rating to "perform urinalysis (e.g. using the dipstick method), including the assessment of white and red blood cells and nitrite, for routine diagnosis" and to "perform urine culture and antimicrobial susceptibility testing in patients with pyelonephritis."

The EAU defines **complicated UTI** (cUTI) as occurring "in an individual in whom factors related to the host (e.g. underlying diabetes or immunosuppression) or specific anatomical or functional abnormalities related to the urinary tract (e.g. obstruction, incomplete voiding due to detrusor muscle dysfunction) are believed to result in an infection that will be more difficult to eradicate than an uncomplicated infection." Other factors associated with cUTIs include vesicoureteral reflux, recent history of instrumentation, UTI in males, pregnancy, and healthcare-associated infections. "Laboratory urine culture is the recommended method to determine the presence or absence of clinically significant bacteriuria in patients suspected of having a cUTI".

For **catheter-associated UTIs** (CAUTI), the EAU recommends with 'Strong' ratings to "not carry out routine urine culture in asymptomatic catheterised patients", to "not use pyuria as sole indicator for catheter-associated UTI", and to "not use the presence or absence of odorous or cloudy urine alone to differentiate catheter-associated asymptomatic bacteriuria from catheter-associated UTI."

In cases of **urethritis,** the EAU states that "Clinicians should always perform point-of-care diagnostics (e.g. Gram staining, first-void urine with microscopy, leukocyte esterase testing) if available to obtain objective evidence of urethral inflammation and to guide treatment…men who meet the criteria for urethritis should be tested for *C. trachomatis, M. genitalium* and *N. gonorrhoea* with nucleic acid amplification tests (NAAT), even if point-of-care tests are negative for gonorrhoeae…*N. gonorrhoeae* and chlamydia cultures are mainly to evaluate treatment failures and monitor developing resistance to current treatment." With a 'Strong' rating, they recommend:

- "Perform a gram stain of urethral discharge or a urethral smear to preliminarily diagnose gonococcal urethritis."

- "Perform a validated nucleic acid amplification tests on a first-void urine sample or urethral smear to prior to empirical treatment to diagnose chlamydial and gonococcal infections."
- "Perform a urethral swab culture, prior to initiation of treatment, in patients with a positive NAAT for gonorrhoea to assess the antimicrobial resistance profile of the infective strain."
- "Use a pathogen directed treatment based on local resistance data."

For **urosepsis,** the EAU strongly recommends to "Take a urine culture and two sets of blood cultures before starting antimicrobial treatment."

For the diagnosis and disease management of **bacterial prostatitis** (BP), the EAU recommends with a 'Strong' rating to "perform the Meares and Stamey 2- or 4-glass test in patients with [chronic bacterial prostatitis (CBP)]". They only give a 'Weak' rating in the use of the urine dipstick test and blood culture with a total blood count for acute bacterial prostatitis (ABP). They also give a 'Weak' rating to their recommendation to "not routinely perform microbiological analysis of the ejaculate alone to diagnose CBP"; however, they give a 'Strong' recommendation to "treat acute bacterial prostatitis according to the recommendations for complicated UTIs" where they recommend a laboratory urine culture.

The EAU's recommendation in cases of suspected **acute infective epididymitis** (with a 'Strong' rating) is "to obtain a mid-stream urine and a first voided urine for pathogen identification by culture and nucleic acid amplification test." It should be noted that, if the acute scrotal pain and/or swelling is due to suspected torsion, then a urine culture is not necessary. In that case, "urgent surgical exploration" is recommended instead. (Boonkat et al., 2023)

World Health Organization (WHO)

The *WHO recommendations on antenatal care for a positive pregnancy experience* in 2016 does include a recommendation to test for asymptomatic bacteriuria (ASB) in pregnant women. "Midstream urine culture is the recommended method for diagnosing asymptomatic bacteriuria (ASB) in pregnancy. In settings where urine culture is not available, the onsite midstream urine Gram-staining is recommended over the use of dipstick tests as the method for diagnosing ASB in pregnancy." They do make note of the amount of time a urine culture takes (up to 7 days) but state that it is "the gold standard". The concern of ASB in pregnancy is because "ASB is associated with an increased risk of preterm birth" (WHO, 2016).

Canadian Paediatric Society (CPS)

In 2014, the CPS issued their position statement titled *Urinary tract infection in infants and children: Diagnosis and management* and reaffirmed their statement in 2020. Their recommendations are for children >2 months old. They recommend that "infants from two to 36 months of age with a fever of >39°C and no other source for fever on history or physical examination…should have urine collected for urinalysis. Unless this test is completely normal, they should then have urine collected by catheter or suprapubic aspirate [SPA] sent for culture." Currently, CPS notes this statement as inapplicable for infants under 2 months of age. (Robinson et al., 2020)

If the child has been toilet-trained, then the urine sample can be collected midstream in lieu of the catheter. "Children with possible UTI who require antibiotic treatment immediately for other indications, such as suspected bacteremia, should have urine collected for urinalysis, microscopy, and culture." Again, this sample should be obtained via either catheterization or SPA unless the child has been toilet-trained. They also state that "urine collection must occur before starting antibiotics because a single dose of an effective antibiotic rapidly sterilizes the urine." (Robinson et al., 2020)

American Academy of Pediatrics (AAP)

The AAP issued guidelines for UTIs in children 2 to 24 months of age in 2011, which were reaffirmed in 2016. With an "A" grade for evidence quality and a strong recommendation, they issued their Action Statement 1: "If a clinician decides that a febrile infant with no apparent source for the fever requires antimicrobial therapy to be administered because of ill appearance or another pressing reason, the clinician should ensure that a urine specimen is obtained for both culture and urinalysis before an antimicrobial agent is administered; the specimen needs to be obtained through catheterization or SPA, because the diagnosis of UTI cannot be established reliably through culture of urine collected in a bag." For instances where the clinician believes that the febrile child does not warrant immediate antimicrobial therapy, the AAP in Action Statement 2 (strong recommendation; "A" grade of evidence) the following: (Action Statement 2a) "If the clinician determines the febrile infant to have a low likelihood of UTI [in Table below] then the clinical follow-up monitoring without testing is sufficient." In Action Statement 2b, the AAP states: "If the clinician determines that the febrile infant is not in a low-risk group [in Table below], then there are 2 choices. Option 1 is to obtain a urine specimen through catheterization or SPA for culture and urinalysis. Option 2 is to obtain a urine specimen through the most convenient means and to perform a urinalysis. If the urinalysis results suggest a UTI (positive leukocyte esterase test results or nitrite test or microscopic analysis results positive for leukocytes or bacteria), then a urine specimen should be obtained through catheterization or SPA and cultures; if urinalysis of fresh (<1 hour since void) urine yields negative leukocyte esterase and nitrite test results, then it is reasonable to monitor the clinical course without initiating anti-microbial therapy, recognizing that negative urinalysis results do not rule out a UTI with certainty." The table below from (Roberts, 2011) depicts the level of risk factors separated by gender.

Individual Risk Factors: Girls
White race Age < 12 mo Temperature ≥ 39°C Fever ≥ 2 d Absence of another source of infection

Probability of UTI	No. of Factors Present
≤1%	No more than 1
≤2%	No more than 2

Individual Risk Factors: Boys
Nonblack race Temperature ≥ 39°C Fever > 24 h Absence of another source of infection

Probability of UTI	No. of Factors Present	
	Uncircumcised	Circumcised
≤1%	[a]	No more than 2
≤2%	None	No more than 3

FIGURE 2
Probability of UTI Among Febrile Infant Girls[28] and Infant Boys[30] According to Number of Findings Present. [a]Probability of UTI exceeds 1% even with no risk factors other than being uncircumcised.

Canadian Urological Association (CUA)

The CUA *Guidelines for the diagnosis and management of recurrent urinary tract infection in women* contains an algorithm for a "female without a prior history of structural or functional abnormalities of the urinary tract presenting with 3 or more UTIs in 12 months" that requires a urine culture during a time when the patient is symptomatic followed by a urine culture two weeks after initiating treatment with sensitivity-adjusted antibiotics (Level 4 evidence, Grade C recommendation [Recommendation 2c]). In doing so, this "may aid in confirming the diagnosis of UTI, as well as guiding further specialist evaluation and management." For recurrent uncomplicated UTI, "culture and sensitivity analysis should be performed at least once while the patient is symptomatic…. A midstream urine bacterial count of 1 X 10^5 CFU/L should be considered a positive culture while the patient is symptomatic." For patients that choose an option of 'self-start antibiotic' therapy, "it is not necessary to culture the urine after UTI self-diagnosis since there is a 86% to 92% concordance between self-diagnosis and urine culture in an appropriately selected patient population. Patients are advised to contact a health care provider if symptoms do not resolve within 48 hours for treatment based on culture and sensitivity." (Dason et al., 2011).

American Urological Association (AUA)

The AUA issued a white paper in 2014 concerning CAUTIs. In the white paper, they refer to the use of the National Surgical Quality Improvement Program (NSQIP) definition of UTIs, which does reference the use of urine culture. It should be noted, however, that this definition requires at least a minimum of one of the following symptoms: fever (>38°C), urgency, frequency, dysuria, or suprapubic tenderness. They, too, refer to the 2009 IDSA guidelines concerning CAUTIs as well as those of the EAU. They state that there are "no consistent guidelines are available on how to obtain urine for culture from chronically catheterized patients, or what constitutes true urinary tract infection versus asymptomatic bacteriuria."

They make note of a study concerning the possible cost-effectiveness of the use of dipsticks to screen asymptomatic ICU patients for CAUTIs. They conclude, "however, as previously discussed, screening of asymptomatic patients may not be warranted, and treatment is usually not recommended in these cases" (Averch et al., 2014).

The AUA released guidelines for primary vesicoureteral reflux in children and recommend "Urinalysis for proteinuria and bacteriuria is recommended. If the urinalysis indicates infection, a urine culture and sensitivity is recommended". The AUA also recommends urinalysis annually as part of the follow-up procedure (AUA, 2017).

The AUA published an update to their 2012 guideline on Urologic Procedures and Antimicrobial Prophylaxis, termed a "Best Practice Statement".

The AUA recommends that "Prior to any urologic procedure, evaluation of a patient's urinary tract symptoms suggestive of a UTI should include a simple dipstick, laboratory performed microscopy, and/or formal culture".

The AUA also states that "Positive microscopy findings should be confirmed with a culture for antimicrobial sensitivities in the perioperative setting where the risk of an SSI is high and targeted antimicrobial treatment may be required. Urine culture should not be performed without an accompanying urine microscopy due to common sample contamination as well as bacterial colonization". (Lightner et al., 2020)

National Institute for Health and Care Excellence (NICE)

In 2023, the NICE updated their quality standards for urinary tract infections in adults. They released five quality statements:

- "Statement 1: Women aged under 65 years are diagnosed with a urinary tract infection (UTI) if they have 2 or more key urinary symptoms and no other excluding causes or warning signs.
- Statement 2: Adults with indwelling urinary catheters do not have dipstick testing to diagnose UTIs.
- Statement 3: Men and non-pregnant women are not prescribed antibiotics to treat asymptomatic bacteriuria.
- Statement 4: Non-pregnant women with an uncomplicated lower UTI are prescribed a 3-day course of antibiotics, and men and pregnant women with an uncomplicated lower UTI are prescribed a 7-day course of antibiotics.
- Statement 5: Men with a recurrent UTI, and women with a recurrent lower UTI where the cause is unknown or a recurrent upper UTI are referred for specialist advice. (NICE, 2023).

NICE also recommended the following populations of children for a urine culture:

- in infants and children who are suspected to have acute pyelonephritis/upper urinary tract infection
- in infants and children with a high to intermediate risk of serious illness
- in infants under 3 months
- in infants and children with a positive result for leukocyte esterase or nitrite

- in infants and children with recurrent UTI
- in infants and children with an infection that does not respond to treatment within 24–48 hours, if no sample has already been sent
- when clinical symptoms and dipstick tests do not correlate (NICE, 2018)

American Urological Association (AUA)/Canadian Urological Association (CUA)/Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction (SUFU)

The AUA, CUA, and SUFU released joint guidelines in 2019. The guidelines were reviewed, and validity confirmed in 2022. These joint guidelines focus on "recurrent episodes of uncomplicated cystitis in women" and are not intended for "pregnant women, patients who are immunocompromised, those with anatomic or functional abnormalities of the urinary tract, women with rUTIs due to self-catheterization or indwelling catheters, or those exhibiting signs or symptoms of systemic bacteremia, such as fever and flank pain". Their recommendations are listed below:

- "Clinicians should obtain urinalysis, urine culture and sensitivity with each symptomatic acute cystitis episode prior to initiating treatment in patients with rUTIs. (Moderate Recommendation; Evidence Level: Grade C)"
- "Clinicians should omit surveillance urine testing, including urine culture, in asymptomatic patients with rUTIs" (Moderate Recommendation; Evidence Level: Grade C)" (Anger et al., 2019)

Infectious Diseases Society of America (IDSA)

- These 2019 guidelines were intended to update the 2005 IDSA guidelines. Their recommendations for asymptomatic bacteriuria (ASB) are as follows:
- "In infants and children, we recommend against screening for or treating asymptomatic bacteriuria".
- "In healthy premenopausal, nonpregnant women or healthy postmenopausal women, we recommend against screening for or treating ASB".
- "In pregnant women, we recommend screening for and treating ASB".
- "In older, community-dwelling persons who are functionally impaired, we recommend against screening for or treating ASB".
- "In older persons resident in long-term care facilities, we recommend against screening for or treating ASB".
- "In patients with diabetes, we recommend against screening for or treating ASB".
- "In renal transplant recipients who have had renal transplant surgery >1 month prior, we recommend against screening for or treating ASB".
- "In patients with nonrenal solid organ transplant (SOT), we recommend against screening for or treating ASB".
- "In patients with high-risk neutropenia (absolute neutrophil count <100 cells/mm3, ≥7 days' duration following chemotherapy), we make no recommendation for or against screening for or treatment of ASB".

- "In patients with spinal cord injury (SCI), we recommend against screening for or treating ASB".
- "In patients with a short-term indwelling urethral catheter (<30 days), we recommend against screening for or treating ASB".
- "In patients undergoing elective nonurologic surgery, we recommend against screening for or treating ASB".
- "In patients who will undergo endoscopic urologic procedures associated with mucosal trauma, we recommend screening for and treating ASB prior to surgery".

The guideline also states that it has been reviewed and endorsed by the following societies: "the Society of Healthcare Epidemiology of America, Pediatric Infectious Diseases Society, American College of Obstetrics and Gynecology, Association of Medical Microbiology and Infectious Diseases Canada, European Society of Clinical Microbiology and Infectious Diseases, European Association of Urology, and the American Urological Association" (USPSTF, 2019).

US Preventive Services Task Force (USPSTF)

The USPSTF recommends screening for "asymptomatic bacteriuria using urine culture in pregnant persons", but recommends against "screening for asymptomatic bacteriuria in nonpregnant adults" (USPSTF, 2019).

American Society of Transplantation Infectious Diseases

These guidelines focus on UTIs within the kidney transplant (KT) population. The recommendations are listed below:

"We recommend against routinely collecting urine culture or treating bacteriuria in asymptomatic KT patients more than two months after KT".

"If screening asymptomatic KT recipients any time in the post-transplant period and AB [asymptomatic bacteriuria] is found, a second urine culture (minimizing risk of contamination) should be collected and reviewed prior to decision about whether or not to treat AB. We strongly recommend observation without treatment of asymptomatic KT patients recipients who show clearance of the initial bacteriuria or development of different organism in the urine" (Goldman & Julian, 2019).

Choosing Wisely Canada

The Association of Medical Microbiology and Infectious Diseases Canada recommends against collecting "urine specimens for culture from adults who lack symptoms localizing to the urinary tract or fever unless they are pregnant or undergoing genitourinary instrumentation where mucosal bleeding is expected." The guideline further recommends that laboratories "consider supplementing educational efforts to reduce collection of urine cultures from asymptomatic patients with analytical interventions that reduce processing of low-value specimens" (Association of Medical Microbiology and Infectious Diseases Canada, 2021).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87086	Culture, bacterial; quantitative colony count, urine
87088	Culture, bacterial; with isolation and presumptive identification of each isolate, urine
87140	Culture, typing; immunofluorescent method, each antiserum
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (eg, antibiotic gradient strip)
87147	Culture, typing; immunologic method, other than immunofluorescence (eg, agglutination grouping), per antiserum
87186	Susceptibility studies, antimicrobial agent; microdilution or agar dilution (minimum inhibitory concentration [MIC] or breakpoint), each multi-antimicrobial, per plate

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
8/24/22	Modified overall coverage criteria to align language with updated clinical standards.

Vectra DA Blood Test for Rheumatoid Arthritis

Policy #: AHS – G2127	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:**

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disorder which results from a complex interaction between genes and the environment, leading to a breakdown of immune tolerance and synovial inflammation in a characteristic symmetric pattern. RA usually leads to the destruction of joints due to erosion of cartilage and bone, causing joint deformities (Firestein, 2020).

Vectra DA is a multi-biomarker disease activity (MBDA) blood test which combines the levels of 12 serum biomarkers into a single score from 1 to 100 to provide an objective measure of RA disease activity. It is intended for use with existing symptom-based disease activity measures to improve long-term outcomes for RA patients (van der Helm-van Mil, Knevel, Cavet, Huizinga, & Haney, 2013).

This policy does not pertain to general inflammation; for guidance on general inflammation testing, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), please see policy AHS-G2155 General Inflammation Testing and policy AHS-G2022 ANA/ENA Testing.

II. Related Policies

Policy Number	Policy Title
AHS-G2022	ANA/ENA Testing
AHS-G2155	General Inflammation Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. The use of a multi-biomarker disease activity score for rheumatoid arthritis (e.g., Vectra DA score) **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Rheumatoid arthritis (RA) affects over 1.3 million people in the U.S. and over 4 million worldwide. Despite the availability of potent biologic treatments, substantial disease activity persists in many patients, with accompanying progressive bone and soft tissue damage, extra-articular consequences, disability, and increased mortality (Centola et al., 2013). The condition usually involves stiff and swollen joints throughout the body, pain, and eventually the destruction of the affected joints. This typically leads to significant motor disability in patients who do not respond to treatment (Venables, 2019).

Measuring disease activity has become important for the management of patients with RA (Curtis et al., 2012). As RA is a chronic illness, earlier and more aggressive treatment may provide significant benefits, especially for patients with more severe forms of the illness (Taylor & Maini, 2020). Tighter control, such as more frequent monitoring and actively striving to meet a disease activity level, has been advantageous in several studies (Bakker, Jacobs, Verstappen, & Bijlsma, 2007; Mease, 2010). However, there is no gold standard for disease activity assessment in RA. Multiple measures are used, and no single measure of disease activity has been recommended in U.S. or international RA guidelines (Centola et al., 2013). Disease activity indices are based on clinical, laboratory, and physical measures. Most of these indices, such as the Disease Activity Score (DAS) and the Routine Assessment of Patient Index Data-3 (RAPID-3), rely on either clinical evaluation of joints, patient-reported outcomes (PROs), or both in disease activity assessment. However, high intra- and inter-observer variability occurs. Furthermore, prior damage to joints or other conditions may influence these measurements (Curtis et al., 2012). Other commonly used tools for diagnosing RA have significant weaknesses; for example, blood tests may be used but are completely normal for many RA patients. MRI may be used due to its ability to identify early signs, but it is expensive and time consuming (Li, Sasso, van der Helm-van Mil, & Huizinga, 2016).

Biologic markers or "biomarkers" can provide objective measurements that reflect underlying pathophysiological processes, pathogenic processes, or responses to treatment. Most measures of monitoring disease and treatment progress rely on subjective measurements, such as joint evaluation,

so biomarkers may be a useful complement in patient management (Taylor & Maini, 2020). Joint damage at the molecular level may be occurring before any clinical signs appear so identifying any indications of disease activity could allow clinical interventions to be taken earlier (Mc Ardle, Flatley, Pennington, & FitzGerald, 2015). Markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are part of clinical measures such as the DAS. However, these two biomarkers are nonspecific; abnormal amounts of these markers may be due to other reasons apart from RA and may be completely normal in patients with RA (Centola et al., 2013; Curtis et al., 2012). This non-specificity is not limited to ESR and CRP. For example, antibodies (usually called rheumatoid factors or RF) produced against immunoglobulin G (IgG) are often tested to diagnose RA, but these antibodies may be produced in response to another rheumatic condition or a separate chronic infection (Shmerling, 2020). RA is a heterogenous condition, and no single biomarker is a reliable predictor of RA disease activity (Mc Ardle et al., 2015). However, the combined assessment of multiple biomarkers, such as through multi-biomarker disease activity (MBDA), may be useful for predicting disease activity and progression (Taylor & Maini, 2020).

Validity and Utility

According to Curtis et al. (2012), the MBDA algorithm (Vectra DA) was developed by screening 396 candidate biomarkers. An algorithm was then created to generate a composite score based on the 12 biomarkers most correlated to RA clinical disease activity which are as follows:

- Interleukin-6 [IL-6]
- Tumor necrosis factor receptor type I [TNFRI]
- Vascular cell adhesion molecule 1 [VCAM-1]
- Epidermal growth factor [EGF]
- Vascular endothelial growth factor A [VEGF-A]
- YKL-40
- Matrix metalloproteinase 1 [MMP-1]
- MMP-3
- CRP
- Serum amyloid A [SAA]
- Leptin
- Resistin

These biomarkers represent several processes related to RA, such as cartilage remodeling and cytokine signaling pathways. A score of ≤29 is considered "low" activity, between 29 and 44 is "moderate" activity, and >44 is "high" activity. The MBDA is intended to provide separate information from a clinical evaluation of joints and should be used as a complement, not as a replacement (Curtis et al., 2012).

This MBDA has been shown to correlate significantly ($r=0.72$; $p<0.001$) with a disease activity score based on the 28-joint Disease Activity Score based on CRP (DAS28-CRP) and has been validated for clinical use as a disease activity marker in RA (Curtis et al., 2012). Both Hirata et al. (2013) and Bakker et al. (2012) found the MBDA score to correlate well with disease activity and could complement other existing measures of RA assessment. Remission based on the MBDA score was a significant predictor of

radiographic non-progression, whereas both remission-defined DAS28-CRP and American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria was not. The MBDA test was also useful in assessing the risk of radiographic progression among patients who met clinical remission criteria. MBDA results may provide an important addition to clinical assessment, however, further studies are needed to confirm its clinical utility in the management of RA (van der Helm-van Mil et al., 2013).

Li, Sasso, Emerling, Cavet, and Ford (2013) evaluated the impact of an MBDA blood test for rheumatoid arthritis (RA) on treatment decisions made by six health care providers (HCPs) in 101 patients. HCPs completed surveys before and after viewing the MBDA test result, recording dosage and frequency for all RA medications and assessment of disease activity. Frequency and changes in treatment plan that resulted from viewing the MBDA test result were determined. The MBDA test results were found to have changed 38% of patients' treatment plans. Furthermore, treatment plans were changed 63% of the time the MBDA test results were found to be "not consistent" or "somewhat consistent" with the clinical assessment of disease activity. However, any improvement in clinical outcomes caused was not reported, and the overall amount of drug use was not affected (Li et al., 2013).

Another study by Li et al. (2016) assessed the correlation between MBDA score and disease progression in 163 RA patients. The study found that low radiographic progression was associated with low MBDA scores and higher scores were associated with more frequent and severe progression. Notably, MBDA scores correlated with progression even when a conventional measure such as the DAS28 indicated otherwise. For example, low risk of progression was associated with a low MBDA score, even when a concurrent DAS28 score was high. The authors concluded that MBDA may be a good complement for conventional measures, as well as provide information on changing treatment plans (Li et al., 2016).

Curtis, Greenberg, Harrold, Kremer, and Palmer (2018) initially studied the influence of age, obesity and other comorbidities on the MBDA test. A cross-sectional analysis of RA patients who have participated in an MBDA test was used (n=357). "Of 357 eligible patients, 76% (n = 273) had normal CRP (<10mg/L) with high (33%), moderate (45%), and low (22%) disease activity by MBDA. The MBDA score was significantly associated with BMI, age, CDAI [clinical disease activity index], and SJC [swollen joint count] (Curtis et al., 2018)." Almost one third of participants had normal CRP scores but high MBDA scores. "In this real-world analysis, the MBDA score was associated with RA disease activity, obesity, and age, and was negligibly affected by common comorbidities (Curtis et al., 2018)."The authors conclude by suggesting that an adjusted MBDA score may require development to account for BMI and age. Such a study was then published the following year. Curtis et al. (2019) developed an MBDA test that will include additional factors such as sex, age and obesity in RA patients. Obesity, or adiposity, was measured using either BMI or serum leptin concentration. Two cohorts were studied, totaling 1736 patients. Overall, the authors have developed "a leptin-adjusted MBDA score that has significantly improved [the] ability to predict clinical disease activity and radiographic progression (Curtis et al., 2019)." It was suggested that this leptin-adjusted MBDA score "significantly adds information to DAS28-CRP and the original MBDA score in predicting radiographic progression. It may offer improved clinical utility for personalized management of RA (Curtis et al., 2019)."

A recent study analyzed the measurement of serum biomarkers at early RA disease onset in hopes to better predict disease progression (Brahe et al., 2019). MBDA score and changes in this score were evaluated to predict DAS28-CRP remission. A total of 180 patients participated in this study and were treated with either methotrexate and adalimumab (n = 89) or methotrexate and placebo (n = 91) in addition to a glucocorticoid injection into swollen joints; results showed that "Early changes in MBDA score were associated with clinical remission based on DAS28-CRP at 6 months (Brahe et al., 2019)."

In a study by Ma et al. (2020), the MBDA test was used to explore the role of biomarkers in predicting remission of RA. Serum samples for 148 patients were assessed for MBDA score at three months, six months, and at one year. RA patients on greater than six months stable therapy in stable low disease activity were assessed every three months for one year. Patients not fulfilling any remission criteria at baseline were classified as 'low disease activity state' (LDAS). Patients not fulfilling any remission criteria over 1 year were classified as 'persistent disease activity' (PDA). Of the 148 patients, 27% were in the LDAS group and over 1 year, 9% of patients were classified as PDA. Baseline MBDA score and concentrations of IL-6, leptin, SAA and CRP were significantly lower in all baseline remission criteria groups in comparison to LDAS groups. The individual MBDA biomarkers (IL-6, leptin, SAA, CRP) and initial MBDA score was able to differentiate between remission at baseline and LDAS. The authors state that these findings highlight the potential value of repeated measurements of MBDA score to evaluate the stability of clinical disease activity over time (Ma et al., 2020).

V. Guidelines and Recommendations

American College of Rheumatology (ACR) (Anderson et al., 2012; Singh et al., 2015)

The ACR convened a Working Group (WG) to evaluate the validity, feasibility, and acceptability of available RA disease activity measures.

The WG recommended the following measures:

- Clinical Disease Activity Index
- Disease Activity Score with 28-joint counts (erythrocyte sedimentation rate or C-reactive protein
- Patient Activity Scale (PAS) and PAS-II
- Routine Assessment of Patient Index Data (3 measures)
- Simplified Disease Activity Index

According to the WG, these measures were recommended because "they are accurate reflections of disease activity; are sensitive to change; discriminate well between low, moderate, and high disease activity states; have remission criteria; and are feasible to perform in clinical settings (Anderson et al., 2012)."

The WG also recognized "there is no ideal measure of disease activity" and acknowledged that some measures excluded in their review may be superior to the six recommended measures. However, they believed they identified the best measures of disease activity in RA (Anderson et al., 2012).

In 2015, the ACR published guidelines for the treatment of RA. While these guidelines focus mainly on methods of treatment rather than types of testing, "The team also discussed the following topics and

recommended that they be targeted for future research: use of biologics and DMARDs during the period of conception, pregnancy, and breastfeeding; treatment of RA with interstitial lung disease; laboratory monitoring for biologics/tofacitinib; and biomarker testing (Singh et al., 2015)."

European League Against Rheumatism (EULAR) (Combe et al., 2017; England et al., 2019; Smolen et al., 2020)

EULAR recommends clinical examination as the method of detecting arthritis, but if a definite diagnosis cannot be reached, other risk factors such as rheumatoid factor or swollen joints should be considered.

EULAR states that the main goal of disease-modifying antirheumatic drugs (DMARDs) is clinical remission and regular monitoring of adverse events, disease activity and comorbidities should occur. Monitoring should include joint counts, patient and physician global assessment, and ESR and CRP measurements. Other measures such as radiographic can complement the main measures.

EULAR notes that several combinations of biomarkers have been evaluated, but not validated. Additionally, EULAR states that current data is not convincing and further study is required (Combe et al., 2017).

A 2019 EULAR update focused on management of RA with DMARDs stated that "The major weakness of our current treatment approaches is the lack of biomarkers for immediate stratification of an individual patient to the most appropriate drug. Importantly, these considerations emphasise the need to search for predictive markers; however, since a considerable number of patients (about 20%–30%) are refractory to all current treatment options, new therapies also need to be developed" (Smolen et al., 2020).

Another 2019 EULAR update focused on use of disease activity measures for RA. In it, the guideline identified 11 measures of RA disease activity that met the authors' "minimum standards for regular use", which was defined to include the following four characteristics:

"1) providing a numerical value,

2) categorizing to ≥3 disease states which separate low, moderate, and high disease activity,

3) being feasible for regular measurement in clinic and

4) possessing adequate psychometric properties".

The guideline identified Vectra DA as one of the 11 disease activity measures fulfilling these four minimum standards. However, the guideline recommended five other measures of disease activity, while giving Vectra an "Inconclusive" rating (England et al., 2019).

National Institute for Care and Excellence (NICE), Quality Standard, Rheumatoid arthritis in over 16s (NICE, 2019, 2020)

NICE recommends monthly monitoring of CRP and disease activity until remission or low disease activity. Remission is defined as a DAS28 score of under 2.6, and low is defined as a DAS28 score of under 3.2. NICE does not mention biomarkers in its recommendations for research (NICE, 2020).

The NICE recently published recommendations regarding laboratory testing for rheumatoid arthritis. These guidelines state that "Enzyme-linked immunosorbent assay (ELISA) tests for therapeutic monitoring of tumour necrosis factor (TNF)-alpha inhibitors (drug serum levels and antidrug antibodies) show promise but there is currently insufficient evidence to recommend their routine adoption in rheumatoid arthritis. The ELISA tests covered by this guidance are Promonitor, IDKmonitor, LISA-TRACKER, RIDASCREEN, MabTrack, and tests used by Sanquin Diagnostic Services (NICE, 2019)."

Treat to Target Task Force (2014 Update to 2010 Guidelines) (Smolen et al., 2016)

The task force states that remission or low disease activity is the goal of treatment. Remission is defined as absence of clinical signs and symptoms of disease activity. The task force was reconvened to update their previously issued guidelines from 2010. The task force recommended regular monitoring and documentation of disease activity. The frequency may depend on activity; for higher disease activity, the frequency may be as high as monthly whereas a lower activity patient may only need be re-evaluated every six months (Smolen et al., 2016).

VI. State and Federal Regulations (as applicable)

There are no U.S. Food and Drug Administration (FDA)-approved multibiomarker disease activity (MBDA) tests for measuring disease activity in RA, Vectra included. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81490	Autoimmune (rheumatoid arthritis) analysis of 12 biomarkers using immunoassays, utilizing serum, prognostic algorithm reported as a disease activity score

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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IX. Revision History

Revision Date	Summary of Changes

Venous and Arterial Thrombosis Risk Testing

Policy #: AHS – M2041	**Prior Policy Name & Number (as applicable):** AHS – M2041 – Venous Thrombosis Risk Testing
Implementation Date: 9/15/21	**Date of Last Revision:** 6/15/22, 10/17/23 (See Section VIII)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for Select Health Commercial, Select Health Advantage® (Medicare/CMS) and Select Health Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

A thrombosis, also known as a blood clot, occurs within blood vessels in the body. The two main types of thrombosis include venous thrombosis, which is when a vein is blocked due to a blood clot, and arterial thrombosis, which is when an artery is blocked due to a blood clot. Thrombophilias refer to hereditary and/or acquired abnormalities of hemostasis that predispose patients to thrombosis (Stevens et al., 2016). The most common presentations of venous thromboembolism (VTE) are deep vein thrombosis (DVT) and pulmonary embolism (PE) (Bartholomew, 2017).

II. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For Select Health Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the Select Health Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For Select Health Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the Select Health Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For individuals without recurrent venous thromboembolism (VTE) risk factors (e.g., surgery, prolonged immobilization, collagen vascular disease, malignancy, certain hematologic disorders), plasma testing for protein C deficiency, protein S deficiency, and antithrombin III deficiency (see Note and Note 2) **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) For individuals less than 50 years of age who have experienced any deep venous thrombosis (DVT).

   b) For individuals who have experienced a DVT in unusual sites (e.g., hepatic, mesenteric, or cerebral veins).

   c) For individuals who have experienced a DVT and who have a strong family history of thrombotic disease.

   d) For individuals who are pregnant or taking oral contraceptives and who have experienced a DVT.

   e) For first- and second-degree relatives (see Note 2) of individuals who have experienced a deep vein thrombosis before 50 years of age.

   f) For women under the age of 50 who smoke and who have suffered a myocardial infarction.

   g) Before the administration of oral contraceptives, targeted testing of individuals with a personal or family history of DVT.

   h) For pediatric individuals who have suffered from a pediatric arterial ischemic stroke.

2. For individuals with warfarin-induced skin necrosis or for infants who develop neonatal purpura fulminans, plasma testing for protein C deficiency and protein S deficiency (see Note 1) **MEETS COVERAGE CRITERIA**.

3. Venous thrombosis risk testing for superficial venous thrombosis (including superficial thrombophlebitis and varicosities) **DOES NOT MEET COVERAGE CRITERIA**.

4. For all situations, activated protein C (aPC) resistance assay **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

5. DVT risk testing as part of a pre-transplant evaluation test **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: Plasma testing for protein C deficiency, protein S deficiency, and antithrombin III deficiency should be performed at least six weeks after the acute thrombotic event and while the patient is not taking anticoagulants. Assays for clotting inhibitors amount and function should be performed prior to any molecular testing.

Note 2: In addition to plasma testing (protein C deficiency, protein S deficiency, antithrombin III deficiency), risk factor testing for individuals suspected of having a hereditary and/or acquired thrombophilia should include genetic testing for Factor V Leiden and Prothrombin gene G20210A mutations.

Note 3: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

III. Scientific Background

A thrombus is "an aggregate of coagulated blood within the vascular system or heart which contains platelets, fibrin, leukocytes, and red blood cells in varying amounts" (Herrmann, 2018). This aggregate of blood can be problematic as it may obstruct normal blood circulation throughout the body and even travel to peripheral areas. The primary manifestations of venous thromboembolisms (VTE) are deep vein thrombosis and pulmonary embolism. These conditions affect an estimated one million individuals in the United States annually (Bartholomew, 2017).

Thrombosis is widely theorized to develop due to Virchow's Triad, which consists of abnormalities in blood flow, a vascular endothelial injury, and alterations in the blood constituents. Changes in any of these characteristics may cause the clot to form (Bauer & Lip, 2022b). For example, sickle red blood cells may cause increased clumping or decreased adhesion to the vessel walls (Byrnes & Wolberg, 2017). There are two main types of thrombosis: venous thrombosis (when a vein is blocked due to a blood clot) and arterial thrombosis (when an artery is blocked due to a blood clot).

A deep vein thrombosis (DVT) refers to a thrombus in a "deep" vein whereas a pulmonary embolism (PE) refers to an obstruction of the pulmonary artery (or one of its branches) by foreign material (Bauer, 2022; Thompson, 2022). DVT of the lower extremities may cause symptoms, such as swelling or edema in the lower extremities, pain, and warmth in the affected area (Bauer, 2022). This thrombus may travel to the lungs (becoming an embolus) and cause a PE. A PE has similar symptoms to DVT but may include pulmonary issues, such as shortness of breath. The risk factors for VTE, PE, and DVT are similar (Thompson, 2022). The two primary categories of risk factors for VTE are hereditary and acquired, and the genetic tendency toward VTE is referred to as inherited thrombophilia. Hereditary risk factors include genetic mutations such as Factor V Leiden (FVL) mutations. The five most common genetic risk factors for VTE are FVL mutations, prothrombin mutations, protein S defect, protein C defect, and antithrombin defect (Bauer & Lip, 2020). Approximately 50–60% of the variance in VTE incidence are attributed to genetic effects (Crous-Bou et al., 2016).

A modified activated partial thromboplastin time (aPTT) assay detects the anticoagulant activity of activated protein C (aPC). FVL mutations cause coagulation factor V to be unresponsive to aPC and initially, these changes were termed "aPC resistance" due to the reduced activity of APC on a modified aPTT assay. A single nucleotide change (G1691A) results in a point mutation of glutamine to arginine at position 506. Approximately 99% of carriers of this mutation are heterozygous, and only 5% of these heterozygotes will experience a VTE in their lifetime. These mutations are often suspected in patients experiencing a VTE at a young age (under 50), a VTE in unusual areas such as a portal vein, or recurrent VTEs (Bauer, 2023). Protein C may also be genetically deficient, but this mutation is only seen in 2-5% of individuals with a VTE (Bauer, 2022). Protein S, a cofactor for the aPC control mechanism, and deficiencies in this protein may also confer additional risk for VTE (K. Bauer, 2021b).

The second most common inherited thrombophilia is the G20210A mutation of prothrombin. This mutation is a gain of function mutation where clotting activity is increased by creating more thrombin and fibrin. The overall prevalence of this mutation is about 2% (K. Bauer, 2021c). Genetic defects of antithrombin (an inhibitor of thrombin) may also occur, but the estimated prevalence of antithrombin defects is only a maximum of 0.2% (K. Bauer, 2021a).

Acquired risk factors or predisposing conditions for thrombosis include a prior thrombotic event, recent major surgery, presence of a central venous catheter, trauma, immobilization, malignancy, pregnancy, the use of oral contraceptives or heparin, myeloproliferative disorders, antiphospholipid syndrome (APS), and a number of other major medical illnesses (Bauer & Lip, 2022b). Patients with acquired hypercoagulability have an increased risk of venous thrombosis, arterial thrombosis, or both; however, there is a low risk of recurrence, regardless of thrombophilia status (Connors, 2017). A rare complication of warfarin treatment, warfarin-induced skin necrosis is commonly due to protein C deficiency, with rare cases of protein S deficiency or PVL having been reported (Bauer & Lip, 2022a).

Risk factors for arterial thrombosis are lesser known. The relationship between FVL and arterial thrombosis is controversial with studies reporting varying results; overall, FVL is not currently considered a major risk factor for arterial thrombosis (Carroll & Piazza, 2018; Kujovich, 2011). Kujovich (2018) states that FVL testing should not be performed on persons with any type of arterial thrombosis including myocardial infarction and stroke in children or adults. It has also been reported that while inherited antithrombin, protein S and protein C deficiencies are important risk factors for venous thrombosis, “they have little or no effect on arterial thrombosis” (Previtali et al., 2011). Further, prothrombin gene mutation is not consistently shown to increase the risk of an arterial thromboembolism, and “There is no association of antithrombin deficiency with arterial thrombosis” (Carroll & Piazza, 2018).

It has been proposed that venous thrombosis risk testing may be beneficial as a pre-transplant evaluation test. However, no studies have been identified suggesting this. The North American Thrombosis Forum (NAFT) states that even though certain genetic conditions predispose a small proportion of the population to the development of blood clots, “few people with thrombophilias develop symptoms”; further, there is no cost-effective, safe or long-term method to prevent a blood clot from forming even if a genetic predisposition is identified (NATF, 2019).

Thrombotic events such as thrombophilia and stroke have become increasingly documented in hospitalized pediatric patients with underlying medical conditions such as prematurity, cancer, and congenital heart disease, but they are rarely identified in healthy children. Furthermore, in most cases of pediatric venous thromboembolism, there exist other underlying risk factors such as indwelling central venous catheter and inherited thrombophilia that are worthy of further investigation. The incidence of venous thromboembolisms is highest in neonates and infants, but there is a second peak recorded in adolescence, coinciding with the use of oral contraceptives. However, as in the case with adults, little to no evidence suggests that the use of venous thrombosis risk testing in children will affect the acute management of venous thromboembolisms. In a study including a total of 271 children with VTE, it was found that the relative frequencies of individually inherited thrombophilias were low—for example, the highest recorded frequency of IT disorders was of Factor V Leiden, occurring in only 5 to 10 percent of

the samples. Moreover, a study of 52 children with thromboembolic events during the acute phase did not urge any changes to acute management, regardless of the result of the test (Raffini et al., 2022).

Venous thrombosis risk testing has also been entertained as a manner of combatting pediatric stroke, which can be characterized in a variety of ways, such as by age and by presentation. Arterial ischemic infarctions are the most common, comprising approximately 80% of all perinatal strokes, and this form of stroke can occur in up to 1 in 3500 of newborns. However, though it would seem reasonable for venous thrombosis risk testing to be employed here, recent prospective case-control studies suggest that routine thrombophilia testing is not warranted. The study showed that conditions associated with thrombophilia rarely coincided with arterial ischemic strokes, and these conditions included, but were not limited to, decreased levels of protein C, protein S, or prothrombin, and genotyping of factor V Leiden (FVL) and factor II (FII, prothrombin) G20210A. Of the 14 parameters examined, 12 showed no difference, including all common thrombophilias examined, with specific mention that FVL and FII were comparable to population norms (Curtis et al., 2017; Ferriero et al., 2019). Subsequent evaluation deemed thrombophilia evaluation in neonates as having limited clinical utility because "levels of protein C, protein S, antithrombin, and factor XI are normally decreased to 30% of adults levels, and these levels only approach adult levels at various time points during childhood". Therefore, the use of thrombophilia testing for these proteins may be misleading in the neonatal period, and MRIs instead should be used to diagnose the thrombosis (Ferriero et al., 2019). Moreover, studies focusing on the roles of thrombophilia, arteriopathy, and cardiac abnormalities in perinatal ischemic stroke find that these risk factors were at best unclear, weakening what predictive power they were believed to contain for even recurrent events after perinatal stroke and leading researchers to conclude that thrombophilia evaluation should rarely be considered in cases of perinatal stroke (Lehman et al., 2017).

While the initial aPTT assays used unaltered plasma (first-generation assays), some versions were neither sensitive nor specific for FVL. Modifications to this test resulted in second generation functional aPC resistance assays that correlate well with the presence of FVL. However, in rare cases, functional assays for aPC resistance can give misleading results (e.g., the presence of a lupis anticoagulant can cause falsely abnormal results in some assays; therapy with a direct thrombin inhibitor or oral factor Za inhibitor can cause falsely normal results). In addition, while FVL can be detected by genetic testing or a second-generation functional coagulation test for aPC resistance, individuals with a positive aPC resistance assay would still need to receive genetic testing to confirm a diagnosis (Bauer, 2023). Due to difficulty with interpretation, a need for confirmatory genetic testing, and the overall declining cost of genetic testing, aPC resistance assays are performed infrequently. When performed, they are simply reported as positive, borderline, or negative (K. A. Bauer, 2021).

Clinical Utility and Validity

A D-dimer assay is a blood test that is used in clinical practice to assist in identifying if a patient has a DVT or PE; this test may also help patients experiencing unprovoked VTE to determine if anticoagulation treatment should continue or halt after initial treatment is complete (Linkins & Takach Lapner, 2017). A D-dimer assay may vary greatly based on the type of antibody used, the method of capture, calibration, and instrumentation. Currently, 30 different assays as available which use 20 different monoclonal antibodies; various studies have reported a broad sensitivity and specificity range for D-dimer assays from 69-97% and 43-99% respectively (Linkins & Takach Lapner, 2017). Hence, all D-dimer assays differ

and need to be validated within the population of interest. Because of this, comparing study results is challenging.

Factor VIII is a blood clotting protein encoded by the F8 gene. A case report by Algahtani and Stuckey (2019) suggests that high factor VIII levels may also assist in risk factor determination for thrombosis or ischemic heart disease. "We conclude that high factor VIII levels are a risk factor for thrombosis, with a greater impact on venous than on arterial thrombosis. However, due to a lack of international consensus on methods for the laboratory testing of factor VIII levels in plasma, we would not currently recommend the measurement of factor VIII levels as part of routine thrombophilia screening (Algahtani & Stuckey, 2019)." This relationship has been shown previously as elevated levels of coagulation factor VIII:C were identified in a retrospective study of 584 first-degree relatives of 177 patients with high coagulation factor VIII:C levels; the researchers found that 40% of first degree relatives also had high VIII:C levels and were at an increased risk for VTE and arterial thrombosis when compared to other first-degree relatives with normal VIII:C levels (Bank et al., 2005).

Lee et al. (2017) performed whole exome sequencing on 64 patients with VTE to assess the types of mutations of inherited thrombophilias. Of these 64 patients, 39 of them were found to have a pathogenic variant or variant of unknown significance (VUS). Further, eight were found to have a Factor V mutation (6 with FVL and 2 with less common mutations), two were found to have a prothrombin G20210A mutation, six were found to have a protein S mutation, two were found to have a protein C mutation, and three were found to have an antithrombin mutation (Lee et al., 2017).

Segal et al. (2009) reviewed the utility of FVL and prothrombin G20210A testing. The authors reviewed 124 articles and concluded that although genetic testing for these two risk factors is very accurate (valid), the clinical utility is lacking due to lack of evidence demonstrating improvement in clinical outcomes (Segal et al., 2009).

Onda studied the clinical utility of a new diagnostic algorithm based on serum D-dimer levels for VTE after hepatectomy. 742 patients who underwent hepatectomy were enrolled in the study and measured for serum D-dimer levels post-op. CT scan was performed for patients who had a D-dimer level of greater than 20 µg/mL. Based on D-dimer and CT scan, VTE was diagnosed in 26 patients and pulmonary embolism (PE) was diagnosed in 18 patients. Multivariate analysis also showed that a resected liver weight of more than 120 grams is a significant predictor of VTE. Overall, "patients who undergo hepatectomy are at high risk for VTE, especially when the resected liver weight is high. The proposed diagnostic algorithm based on serum D-dimer levels for VTE after hepatectomy can be useful for early diagnosis" (Onda et al., 2021).

Analytical Validity

Murphy and Sabath (2019) have compared the accuracy and reliability of two tests: a genotypic assay which identifies FVL mutations, and a phenotypic activated protein C resistance assay. Data from 1596 patients was analyzed; each patient had received both types of testing. The authors state that the phenotypic testing exhibited both high sensitivity and specificity compared to genotypic testing. "Phenotypic assays had close to total concordance with genotypic assays over 16 years of testing. Changing ordering practices could result in up to an 80% reduction in testing costs (Murphy & Sabath, 2019)."

A systematic review and meta-analysis by Chiasakul et al. (2019) researched the relationship between inherited thrombophilia and the risk of arterial ischemic stroke in adults. Inherited thrombophilias included FVL, protein C and S deficiency, antithrombin deficiency and prothrombin G20210A mutation. For this study, 11,916 stroke patients and 96,057 controls were identified. The authors concluded that "Compared with controls, patients with arterial ischemic stroke were significantly more likely to have the following inherited thrombophilias: factor V Leiden (OR, 1.25; 95% CI, 1.08-1.44; I2=0%), prothrombin G20210A mutation (OR, 1.48; 95% CI, 1.22-1.80; I2=0%), protein C deficiency (OR, 2.13; 95% CI, 1.16-3.90; I2=0%), and protein S deficiency (OR, 2.26; 95% CI, 1.34-3.80; I2=8.8%) (Chiasakul et al., 2019)." Antithrombin deficiency did not reach statistical significance in this study. Hence, in this review, inherited thrombophilias were found to be associated with an increased risk of arterial ischemic stroke in adults.

In a systematic review, Ortega studied the predictive value of D-dimer testing for venous thrombosis diagnosis in unusual locations. 3378 patients from 23 articles with thrombosis in unusual sites, such as upper extremity deep vein thrombosis (DVT), cerebral vein thrombosis (CVT) and splanchnic vein thrombosis (SVT), were studied. 12 articles on CVT concluded that timing of D-dimer testing is important and patients with short duration of symptoms displayed higher D-dimer levels. Sensitivity and specificity in these patients ranged from 58% to 97% and from 77% to 97.5%, respectively. The authors conclude that "D-dimer testing should not be currently recommended for the diagnosis of thrombosis in unusual sites as a first line diagnostic tool. The development of algorithms combining biomarkers such as D-dimer and clinical decision tools could improve the diagnosis" (Ordieres-Ortega et al., 2020).

IV. Guidelines and Recommendations

American Heart Association/American Stroke Association (AHA/ASA)

The AHA/ASA has issued a scientific statement for the management of stroke in neonates and children, wherein testing for thrombophilic abnormalities are discussed. The AHA/ASA admits that due to the lack of "an adequately powered study to detect the impact of genetic thrombophilia on recurrence risk in pediatric AIS [arterial ischemic stroke], definite recommendations about evaluation remain challenging", but acknowledges that "laboratory testing outside of clinical studies may provide guidance for long-term management of the patient". For cases of thrombophilia the AHA/ASA provides an algorithm for the "Targeted Evaluation of a Child With AIS for Rare Causes or Causes Requiring Additional Evaluation" that includes the examination of Factor VIII level Lipoprotein(a), *MTHFR* mutation, and homocysteine levels, and it is suggested that "non-DNA testing may need to be repeated when the child is older to ensure that adult levels of proteins have been attained" and "measurement of proteins or homocysteine levels in the acute phase of stroke may not be accurate and should be repeated after the acute event". Finally, for the evaluation of a child with AIS, it is believed that "A thrombophilia evaluation is helpful in every case of childhood stroke, especially if there is no identifiable cause, medical history of thrombosis, or a first-degree relative with thrombosis history" (Ferriero et al., 2019).

In 2021, the AHA released guidelines on stroke prevention. The AHA brushes on testing for hematologic traits in the context of secondary stroke prevention. "If in certain clinical scenarios (eg, paradoxical emboli caused by venous thrombosis or recurrent venous thrombosis) testing for thrombophilic states is considered, testing for protein C, protein S, or antithrombin levels should be deferred or repeated at least

4 to 6 weeks (or up to 6 months for factor VIII609) after the acute stroke given that these protein levels may be altered during the acute stroke phase" (Kleindorfer et al., 2021).

American College of Medical Genetics and Genomics (ACMG)

ACMG has released guidelines for laboratory testing of venous thromboembolism (VTE). This 2018 edition superseded the 2005 edition. The guidelines are as follows:

Testing for factor V Leiden and factor II c.*97G>A (this mutation is also known as G20210A) is recommended in the following circumstances:

- A first unprovoked VTE, especially <50 years old
- VTE at unusual sites (such as hepatic portal, mesenteric, and cerebral veins)
- Recurrent VTE
- Personal history of VTE with (a) two or more family members with a history of VTE or (b) one first-degree relative with VTE at a young age
- Patients with low activated protein C (APC) resistance activity

Testing may be considered in the following circumstances:

- Females under the age of 50 who smoke tobacco and have a history of acute myocardial infarction
- Siblings of individuals known to be homozygous for factor V Leiden or factor II c.*97G>A, because they have a 1 in 4 chance of being a homozygote
- Asymptomatic pregnant female or female contemplating pregnancy, with a first-degree relative with unprovoked VTE or VTE provoked by pregnancy or contraceptive use
- Pregnant female or female contemplating pregnancy or estrogen use who has a first-degree relative with a history of VTE and is a known carrier for factor V Leiden and/or factor II c.97*G>A variant
- Pregnant female or female contemplating pregnancy with a previous non-estrogen-related VTE or VTE provoked by a minor risk factor, because knowledge of the factor V Leiden or factor II c.*97G>A status may alter pregnancy related thrombophylaxis (Zhang et al., 2018).

The ACMG found several clinical scenarios requiring special considerations worth mentioning, involving different populations. One involved the testing of asymptomatic versus symptomatic individuals, in which they assert that "It is generally not recommended to test asymptomatic minors as VTE rarely occurs before young adulthood even in the homozygous state." For prenatal testing and population screening, the ACMG suggests that "prenatal testing and population screening are not indicated due to the low penetrance of these variants, later age of onset, and lack of genotype-directed prophylaxis". Lastly, in women considering taking estrogen-containing oral contraceptives (OC) or hormone replacement therapy (HRT), the ACMG indicates that "A family and personal history of thrombosis should be carefully evaluated for all women before initiating HRT and a positive history may warrant thrombophilia screening" (Zhang et al., 2018).

ACMG does not support testing for *MTHFR* variants in thrombophilia assessment due to the lack of correlation with negative pregnancy outcomes (Hickey, Curry, & Toriello, 2013)

American Society of Hematology (ASH)

The 2013 ASH recommends against testing "for thrombophilia in adult patients with venous

thromboembolism (VTE) occurring in the setting of major transient risk factors (surgery, trauma or prolonged immobility) (ASH, 2013)."

In 2018, ASH released their guidelines for management of venous thromboembolism, which included the following recommendations (Lim et al., 2018):

- "*Recommends* using a strategy starting with D-dimer for excluding PE in a population with low prevalence/PTP (≤5%), followed by ventilation-perfusion (VQ) scan or computed tomography pulmonary angiography (CTPA) for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against additional testing following negative CTPA or normal VQ scan in a population with low prevalence/PTP (≤5%).
- S*uggests* using a strategy starting with D-dimer for excluding PE in a population with intermediate prevalence/PTP (~20%), followed by VQ scan or CTPA for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against additional testing following negative CTPA or normal VQ scan in a population with intermediate prevalence/PTP (~20%).
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against using D-dimer as a subsequent test following a negative CT scan in a population with high prevalence/PTP (≥50%).
- *Suggests* using a strategy starting with D-dimer for excluding recurrent PE in a population with unlikely PTP.
- *Recommends* using a strategy starting with D-dimer for excluding DVT in a population with low prevalence/PTP (≤10%), followed by proximal lower extremity ultrasound or whole-leg ultrasound for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose DVT, and against additional testing following negative proximal or whole-leg ultrasound in a population with low prevalence/PTP (≤10%).
- *Recommends against* using a positive D-dimer alone to diagnose DVT in a population with intermediate prevalence/PTP (~25%).
- *Recommends against* using a positive D-dimer alone to diagnose DVT in a population with high prevalence/PTP (≥50%).
- *Suggests* using a strategy starting with D-dimer for excluding recurrent DVT in a population with unlikely PTP.
- *Suggests* a strategy starting with D-dimer for excluding upper extremity DVT in a population with low prevalence/unlikely PTP (10%), followed by duplex ultrasound if D-dimer is positive.
- *Recommends against* using a positive D-dimer alone to diagnose upper extremity DVT in a population with low prevalence/unlikely PTP (10%).
- *Suggests* a strategy of either D-dimer followed by duplex ultrasound/serial duplex ultrasound, or duplex ultrasound/serial duplex ultrasound alone for assessing patients suspected of having upper extremity DVT in a population with high prevalence/likely PTP (40%).
- *Recommends against* using a positive D-dimer alone to diagnose upper extremity DVT in a population with high prevalence/likely PTP (40%) (Lim et al., 2018)."

Society for Vascular Medicine (SVM)

This society recommends against workup for clotting disorders for patients with DVT as treatment will not change based on any abnormalities (SVM, 2013).

American College of Obstetricians and Gynecologists (ACOG)

The 2013 ACOG clinical management guidelines recommend that screening for inherited thrombophilia "may be considered in the following clinical settings:

1. "A personal history of venous thromboembolism that was associated with a non-recurrent risk factor
2. A first degree relative (parent or sibling) with a history of high-risk thrombophilia (ACOG, 2013)."

The 2018 ACOG Practice Bulletin Summary Number 197 supersedes the above 2013 guidelines (Practice Bulletin Number 138). In this update, the ACOG makes the following recommendations regarding screening based on "limited or inconsistent scientific evidence":

"Screening for inherited thrombophilias is not recommended for women with a history of fetal loss or adverse pregnancy outcomes including abruption, preeclampsia, or fetal growth restriction because there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low molecular-weight heparin prevents recurrence in these patients."

"Because of the lack of association between either heterozygosity or homozygosity for the *MTHFR* C677T polymorphism and any negative pregnancy outcomes, including any increased risk of VTE, screening with either *MTHFR* mutation analyses or fasting homocysteine levels is not recommended."

The 2018 ACOG recommends the following screening guideline based on "consensus and expert opinion":

"Among women with personal histories of VTE, recommended screening tests for inherited thrombophilias should include factor V Leiden mutation; prothrombin G20210A mutation; and antithrombin, protein S, and protein C deficiencies." (ACOG, 2018)

Evaluation of Genomic Applications in Practice and Prevention (EGAPP)

"The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group found adequate evidence to recommend against *routine* testing for Factor V Leiden (FVL) and/or prothrombin 20210G>A (PT) in the following circumstances: (1) adults with idiopathic venous thromboembolism (VTE). In such cases, longer term secondary prophylaxis to avoid recurrence offers similar benefits to patients with and without one or more of these mutations. (2) Asymptomatic adult family members of patients with VTE and an FVL or PT mutation, for the purpose of considering primary prophylactic anticoagulation. Potential benefits are unlikely to exceed potential harms. The evidence was insufficient to determine whether FVL/PT testing might have clinical utility in some circumstances, such as for identifying FVL homozygosity among asymptomatic family members of adults with idiopathic VTE or counseling patients about the risks and benefits of antithrombotic therapy. The recommendations do not extend to patients with other risk factors for thrombosis, such as contraceptive use, as the evidence review that serves as the basis for the recommendations focused primarily on idiopathic VTE (EGAPP, 2011)."

The Anticoagulation Forum

The Anticoagulation Forum published guidance in the Journal of Thrombosis and Thrombolysis on (Stevens et al., 2016):

- "Do not perform thrombophilia testing following an episode of provoked VTE. A positive thrombophilia evaluation is not a sufficient basis to offer extended anticoagulation following an episode of provoked VTE.
- Do not perform thrombophilia testing in patients following an episode of unprovoked VTE. If a patient with unprovoked VTE and low bleeding risk is planning to stop anticoagulation, test for thrombophilia if test results would change this decision. A negative thrombophilia evaluation is not a sufficient basis to stop anticoagulants following an episode of unprovoked VTE in a patient with low bleeding risk and willingness to continue therapy.
  Heterozygosity for FVL or PGM does not increase the predicted risk of recurrence after unprovoked VTE to a clinically significant degree.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia. As a family history of VTE confers an excess risk of thrombosis, relatives should be counseled regarding use of prophylaxis in high risk situations.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia who are contemplating use of estrogen. If a woman contemplating estrogen use has a first-degree relative with VTE and a known hereditary thrombophilia, test for that thrombophilia if the result would change the decision to use estrogen.
- Do not perform thrombophilia testing at the time of VTE diagnosis or during the initial 3-month course of anticoagulant therapy. When testing for thrombophilias following VTE, use either a 2-stage testing approach or perform testing after a minimum of 3 months of anticoagulant therapy has been completed, and anticoagulants have been held.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia who are contemplating pregnancy. If a woman contemplating pregnancy has a first-degree relative with VTE and a known hereditary thrombophilia… test for that thrombophilia if the result would change VTE prophylaxis decisions (Stevens et al., 2016)."

American College of Cardiology (ACC)

In 2017 guidance published in the New England Journal of medicine by Gupta was summarized by Barnes for the American College of Cardiology:

1. "Venous thromboembolism (VTE) affects an estimated 300,000-600,000 patients annually in the United States.
2. The risk of VTE recurrence is best predicted by whether the initial VTE episode was provoked or unprovoked, not the results of inherited thrombophilia testing.
3. Most patients with a provoked VTE have recently undergone surgery, immobility, trauma, or have a concurrent cancer diagnosis. Concurrent use of hormones (e.g., estrogen-containing contraceptive pills) is also frequently considered a provoking factor for VTE development.
4. For patients with a first provoked VTE event, guidelines recommend anticoagulation for only 3 months (not longer). Prolonged anticoagulation is associated with an increased risk of bleeding that outweighs the risk of VTE recurrence for these patients.

5. Patients with an unprovoked VTE (none of the provoking risk factors listed above) require longer anticoagulation due to a higher risk of recurrence that outweighs the risk of bleeding associated with long-term anticoagulation therapy.
6. Thrombophilia testing performed in the setting of an acute clot or ongoing anticoagulation therapy will often result in spurious results (usually false positive). For example, natural anticoagulants (e.g., protein C and S, antithrombin) are consumed during an acute thrombotic event and the levels can be reduced by ongoing anticoagulant therapy.
7. A recent study identified that up to 55% of Medicare patients with provoked VTE had undergone inappropriate thrombophilia testing, associated with significant cost to the healthcare system.
8. While thrombophilia testing rarely impacts management decisions about anticoagulation therapy, it may be beneficial for genetic testing purposes in patients presenting with a first unprovoked VTE at a young age (e.g., < 45 years) or at an unusual site.
9. For patients with unprovoked VTE at a young age, VTE at an unusual site, arterial thrombosis, or pregnancy morbidity, testing for antiphospholipid antibodies, JAK2 mutation, and paroxysmal nocturnal hemoglobinuria may be beneficial.
10. There is no role for extensive cancer screening (e.g., computed tomography scanning) in patients with VTE. Only routine, age-appropriate cancer screening is recommended (G. Barnes, 2017; Gupta et al., 2017)."

Again in 2017, key points—inclusive of guiding points—published in the New England Journal of Medicine by Connors were captured by Barnes for the American College of Cardiology:

1. "The majority of patients with venous thromboembolism (VTE) should not be tested for thrombophilia. Data supporting clinical usefulness and benefits are limited or nonexistent.
2. Most patients with inherited thrombophilia can be identified by coagulation experts based on the patient's personal and family history of VTE. Thrombophilia testing is usually not required.
3. Factors associated with an inherited thrombophilia include VTE at a young age (<40-50 years), a strong family history of VTE, VTE in conjunction with weak provoking factors at a young age, recurrent VTE, and VTE in an unusual site (e.g., cerebral or splanchnic veins).
4. Do not perform thrombophilia testing at the time of a VTE event, as it can be inaccurate (often false positive). Perform testing (when indicated) after completion of initial therapy and if it might change management strategies.
5. Do not perform thrombophilia testing while a patient is receiving anticoagulation. Instead, wait until 2 weeks after discontinuing warfarin, or 2 days for direct oral anticoagulants and heparin.
6. The goal of thrombophilia testing should be to aid decision making regarding future VTE prophylaxis, to guide testing of family members, and to determine the cause in severe or fatal VTE. Test results alone should not be used to decide on the duration of anticoagulation therapy.
7. Most VTE recurrence risk tools do not incorporate thrombophilia test results into their risk stratification schemes.
8. For patients with provoked VTE, even if they have homozygous factor V Leiden, prothrombin gene mutations, or deficiencies of protein S, C, or antithrombin, they do not require lifelong anticoagulation.

9. Currently available thrombophilia tests are insufficient to identify inherited risks of VTE. Therefore, a negative test should not be interpreted as a patient being free of thrombophilia.
10. Testing for the antiphospholipid antibody syndrome may be useful in patients with unprovoked VTE if there is clinical equipoise about extended anticoagulation courses. It can also be useful to determine warfarin versus direct oral anticoagulant therapy (G. D. Barnes, 2017; Connors, 2017)."

American Society for Clinical Pathology (ASCP)

ASCP has published guidelines with Choosing Wisely which state: "Do not test for Protein C, Protein S, or Antithrombin (ATIII) levels during an active clotting event to diagnose a hereditary deficiency because these tests are not analytically accurate during an active clotting event. These assays may be useful to test for an acquired deficiency (i.e., disseminated intravascular coagulation) in consumptive coagulopathies. These tests are not analytically accurate during an active clotting event. Moreover, they are not clinically actionable at the time of an acute clot, because the same therapeutic intervention (anticoagulation) is performed regardless of the results. Deferral to the outpatient/non-acute setting allows for the testing to be done at a time when the results would change patient management, i.e., ceasing or continuing anticoagulation. Because anticoagulation may also impact the determination of results (e.g., Protein C and Protein S decrease on warfarin, while ATIII is actually elevated), testing while on anticoagulants may also yield misleading results and should be avoided(ASCP, 2017)."

In 2019, an additional guideline was put forth by the ASCP on Choosing Wisely: "Do not perform a hypercoagulable workup in patients taking direct factor Xa or direct thrombin inhibitors." The guideline explained that the use of certain direct oral anticoagulants may render the results of such workups uninterpretable and inaccurate (ASCP, 2019).

European Society of Cardiology (ESC)

The ESC has published guidelines for the diagnosis and management of acute PE. These guidelines state:

- "D-dimer measurement and clinical prediction rules should be considered to rule out PE during pregnancy or the post-partum period
- Plasma D-dimer measurement, preferably using a highly sensitive assay, is recommended in outpatients/emergency department patients with low or intermediate clinical probability, or those that are PE-unlikely, to reduce the need for unnecessary imaging and irradiation
- A D-dimer test, using an age-adjusted cut-off or adapted to clinical probability, should be considered as an alternative to the fixed cut-off level
- D-dimer measurement is not recommended in patients with high clinical probability, as a normal result does not safely exclude PE, even when using a highly sensitive assay
- Assessment of the RV [right ventricle] by imaging methods or laboratory biomarkers should be considered, even in the presence of a low PESI [Pulmonary Embolism Severity Index] or a negative sPESI [simplified Pulmonary Embolism Severity Index] (Konstantinides et al., 2019)."

In 2021, the ESC Working Group released guidelines on diagnosis and management of acute deep vein thrombosis. These guidelines suggest that "ELISA D-dimer or highly sensitive immunoturbidimetric tests should be measured in 'unlikely' clinical probability patients to exclude DVT diagnosis" (Mazzolai et al., 2022).

Scottish Intercollegiate Guidelines Network (SIGN)

Regarding laboratory tests in the assessment of thrombosis risk, SIGN has stated that "Routine laboratory screening for heritable thrombophilias is not recommended (SIGN, 2014)."

American Society for Clinical Laboratory Science (ASCLS)

In 2021, ASCLS published guidelines on Choosing Wisely to suggest against ordering a homocysteine assay as part of the thrombophilia work up. "An elevated homocysteine level is not a clotting disorder and should not be included in thrombophilia testing panels" (ASCLS, 2021).

V. Applicable State and Federal Regulations

A search for "thrombosis" on the FDA website yielded 12 results on January 26, 2021 (FDA, 2021). Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
85300	Clotting inhibitors or anticoagulants; antithrombin III, activity
85301	Clotting inhibitors or anticoagulants; antithrombin III, antigen assay
85302	Clotting inhibitors or anticoagulants; protein C, antigen
85303	Clotting inhibitors or anticoagulants; protein C, activity
85305	Clotting inhibitors or anticoagulants; protein S, total
85306	Clotting inhibitors or anticoagulants; protein S, free
85307	Activated Protein C (APC) resistance assay

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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VIII. Revision History

Revision Date	Summary of Changes
6/15/22	Added coverage criteria #2 and #3.
10/17/23	The following changes were implemented: Addition of new coverage criteria #2: "2) For individuals with warfarin-induced skin necrosis or for infants who develop neonatal purpura fulminans, plasma testing for protein C deficiency and protein S deficiency (see Note 1) **MEETS COVERAGE CRITERIA**." Addition of new coverage criteria #4: "4) For all situations, activated protein C (aPC) resistance assay **DOES NOT MEET COVERAGE CRITERIA**.". Addition of new Note 2: "Note 2: In addition to plasma testing (protein C deficiency, protein S deficiency, antithrombin III deficiency), risk factor testing for individuals suspected of having a hereditary and/or acquired thrombophilia should include genetic testing for Factor V Leiden and Prothrombin gene G20210A mutations."

Vitamin B12 and Methylmalonic Acid Testing

Policy #: AHS –G2014	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 2/20/23 (See Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Vitamin B12, also known as cobalamin, is a water-soluble vitamin required for proper red blood cell formation, key metabolic processes, neurological function, and DNA regulation and synthesis. Hematologic and neuropsychiatric disorders caused by a deficiency in B12 can often be reversed by early diagnosis and prompt treatment (Oh & Brown, 2003).

Methylmalonic acid (MMA) is produced from excess methylmalonyl-CoA that accumulates when Vitamin B12 is unavailable and is considered an indicator of functional B12 deficiency (Sobczynska-Malefora et al., 2014).

Holotranscobalamin (holoTC) is the metabolically active fraction of B12 and is an emerging marker of impaired vitamin B12 status (Langan & Goodbred, 2017).

II. Related Policies

Policy Number	Policy Title
AHS-G2154	Folate Testing
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. For individuals with the following signs and symptoms of Vitamin B12 deficiency, Vitamin B12 testing **MEETS COVERAGE CRITERIA:**
    a. Cutaneous
        i. Hyperpigmentation
        ii. Jaundice
        iii. Vitiligo
    b. Gastrointestinal
        i. Glossitis
    c. Hematologic
        i. Anemia (macrocytic, megaloblastic
        ii. Leukopenia
        iii. Pancytopenia
        iv. Thrombocytopenia
        v. Thrombocytosis
    d. Neuropsychiatric
        i. Areflexia
        ii. Cognitive impairment (including dementia-like symptoms and acute psychosis)
        iii. Gait abnormalities
        iv. Irritability
        v. Loss of proprioception and vibratory sense
        vi. Olfactory impairment
        vii. Peripheral neuropathy
2. For individuals undergoing treatment for Vitamin B12 deficiency, Vitamin B12 testing (performed no sooner than 3 months after initiation of therapy) **MEETS COVERAGE CRITERIA.**
3. Screening for Vitamin B12 deficiency **MEETS COVERAGE CRITERIA** for individuals with one or more of the following risk factors:
    a. For individuals with decreased ileal absorption due to:
        i. Crohn disease
        ii. Ileal resection
        iii. Tapeworm infection
        iv. Having undergone, or for those who have been scheduled for, bariatric procedures such as Roux-en-Y gastric bypass, sleeve gastrectomy, or biliopancreatic diversion/duodenal switch

b. For individuals with decreased intrinsic factor due to:
    i. Atrophic gastritis
    ii. Pernicious anemia
    iii. Postgastrectomy syndrome
    i. For individuals with transcobalamin II deficiency
c. For individuals with inadequate B12 intake:
    i. Due to alcohol abuse
    ii. In individuals older than 75 years or elderly individuals being evaluated for dementia
    iii. In vegans or strict vegetarians (including exclusively breastfed infants of vegetarian/vegan mothers)
    iv. Due to an eating disorder
d. For individuals with prolonged medication use:
    i. Histamine H2 blocker use for more than 12 months
    ii. Metformin use for more than four months
    iii. Proton pump inhibitor use for more than 12 months

4. In asymptomatic high-risk individuals with low-normal levels of vitamin B12 or when vitamin B12 deficiency is suspected but the serum vitamin B12 level is normal or low-normal, methylmalonic acid testing to confirm vitamin B12 deficiency **MEETS COVERAGE CRITERIA.**
5. For the evaluation of inborn errors of metabolism, methylmalonic acid testing **MEETS COVERAGE CRITERIA.**
6. In healthy, asymptomatic individuals, screening for Vitamin B12 deficiency **DOES NOT MEET COVERAGE CRITERIA.**
7. For the confirmation of vitamin B12 deficiency, homocysteine testing **DOES NOT MEET COVERAGE CRITERIA.**
8. Holotranscobalamin testing **DOES NOT MEET COVERAGE CRITERIA** for the screening, testing, or confirmation of vitamin B12 deficiency.

IV. Scientific Background

Vitamin B12 cannot be synthesized by human cells (Means Jr & Fairfield, 2021); rather, it is obtained from animal-derived dietary sources, such as meat, eggs, and dairy products (Hunt et al., 2014), as well as fortified cereals and supplements (Zeuschner et al., 2013). Vitamin B12 deficiency is classically caused by pernicious anemia; however, with modern fortification of western diets, this condition now accounts for only a minority of cases and currently occurs most often due to malabsorption (Means Jr & Fairfield, 2021).

The prevalence of vitamin B12 deficiency in the United States and United Kingdom is approximately 6% in persons younger than 60 years, reaching 20% in those older than 60 years. On the contrary, the prevalence is approximately 40% in Latin America, 70% in Kenyan school children, 80% in East Indian preschool-aged children, and 70% in East Indian adults (Hunt et al., 2014). Risk factors for deficiency include: decreased ileal absorption (Crohn disease, ileal resection, tapeworm infection), decreased intrinsic factor (atrophic gastritis, pernicious anemia, post-gastrectomy syndrome), genetic defects (transcobalamin II deficiency), inadequate intake (alcohol abuse, patients older than 75 years, vegans, or strict vegetarians), prolonged medication use (histamine H2 blocker use for more than 12 months, metformin use for more than four months, proton pump inhibitor use for more than 12 months) (Langan & Goodbred, 2017).

Vitamin B12 plays an essential role in nucleic acid synthesis. Deficiency can result in cell cycle arrest in the S phase or cause apoptosis (Green, 2017) and ultimately bone marrow failure and demyelinating nervous system disease (Stabler, 2013). Vitamin B12 is also critical in the remethylation of homocysteine (Hcy), and deficiency in Vitamin B12 can lead to hyperhomocysteinemia, a condition that has been associated with various cancers, such as breast and ovarian cancers, as well as Parkinson disease (Fan et al., 2020; Hama et al., 2020).

Clinical manifestations of Vitamin B12 deficiency vary in their presence and severity from mild fatigue to severe neurologic impairment (Langan & Goodbred, 2017). Mild deficiency can present as fatigue and anemia with an absence of neurological features. Moderate deficiency may include obvious macrocytic anemia with some mild or subtle neurological features. Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy.

Vitamin B12 deficiency can also cause glossitis and other gastrointestinal symptoms that vary with underlying diseases, such as inflammatory bowel disease or celiac disease (Means Jr & Fairfield, 2021). Early detection and correction of vitamin B12 deficiency with supplementation prevents progression to macrocytic anemia, elevated homocysteine (Hcy), potentially irreversible peripheral neuropathy, memory loss, and other cognitive deficits (Sobczynska-Malefora et al., 2014).

Analytical Validity

Both the clinical recognition of vitamin B12 deficiency and confirmation of the diagnosis by means of testing can be difficult. Several laboratory measures reflecting physiological, static, and functional B12 status have been developed (Hunt et al., 2014); however, there is no universally agreed upon gold standard assay for determining cobalamin levels in humans. The current convention is to estimate the abundance of vitamin B12 using total serum vitamin B12, despite the low sensitivity of this test (Sobczynska-Malefora et al., 2014). Two reportedly highly sensitive vitamin B12 deficiency markers are elevated levels of serum homocysteine and methylmalonic acid, but testing is expensive, and many other conditions may cause an elevation in these markers, including familial hyperhomocysteinemia, folate deficiency, levodopa therapy, and renal insufficiency (Langan & Zawistoski, 2011). Serum methylmalonic acid levels tend to be just as sensitive but more specific than serum homocysteine levels in regards to vitamin B12 deficiency testing, highlighting the former as the preferred testing method by many (Langan & Zawistoski, 2011).

An in-depth meta-analysis by Willis et al. (2011) of serum cobalamin testing included data from 54 different studies. The variability for sensitivity and specificity across the different studies ranged from

13% to 75% for sensitivity and 45% to 100% for specificity, depending on the reference standard used. Researchers conclude that "from the available evidence, diagnosis of conditions amenable to cbl [vitamin B12] supplementation on the basis of cbl [vitamin B12] level alone cannot be considered a reliable approach to investigating suspected vitamin deficiency" (Willis et al., 2011). The test measures total serum cobalamin including both serum holohaptocorrin and serum holotranscobalamin, which may mask true deficiency or falsely imply a deficient state (Hunt et al., 2014).

Vitamin B12 deficiency is present in both infant and pregnant female populations, and monitoring vitamin B12 levels is important in determining maternal and fetal health and growth. Low vitamin B12 levels during pregnancy are associated with a greater risk of preterm birth (Rogne et al., 2017). It seems that current pregnancy-specific cutoffs for vitamin B12 biomarkers are inadequate in the medical field (Schroder et al., 2019). Recently, a new study has identified a novel cutoff value in the vitamin B12 serum of newborns; the B12-related metabolite known as homocysteine (Hcy) is now recommended to have a cutoff value at "4.77 µmol/L (68.4% sensitivity, 58.3% specificity, p = .012) for the detection of vit-B12 deficiency" (Yetim et al., 2019). Other pregnancy-specific B12 biomarkers have been published. According to another study, "The central 95% reference interval limits indicated that serum total B-12 <89.9 and <84.0 pmol/L, holoTC <29.5 and <26.0 pmol/L and MMA >371 and >374 nmol/L, in the first and second trimesters, respectively, may indicate B-12 deficiency in pregnant women. The lower limits of total B-12 and holoTC and the upper limits of MMA significantly differed by ethnicity in both trimesters. According to the change point analysis, total B-12 <186 and <180 pmol/L and holoTC <62.2 and <67.5 pmol/L in the first and second trimesters, respectively, suggested an increased probability of impaired intracellular B-12 status, with no difference between ethnicities (Schroder et al., 2019)."

Elevated levels of downstream metabolites, MMA and Hcy, are commonly used as adjuvant diagnostics to confirm a suspected diagnosis of cobalamin deficiency (Berg & Shaw, 2013). The sensitivity of elevated serum MMA measurements in detecting patients with overt cobalamin deficiency is reported to be >95%; however, the specificity of this test has not been determined (Hunt et al., 2014). In a study by Rozmarič et al. (2020) the cutoff for MMA as an indicator of B12 deficiency was 0.423 µM with a specificity of 0.90 and sensitivity of 0.91 in newborns; "applying a screening algorithm including only tHCy [total homocysteine] as a second-tier test that may be feasible for many newborn screening labs, newborns with low VitB12, low HoloTC, or elevated MMA can be identified with a positive predictive value between 59% and 87%."

Serum holoTC may be a better indicator of B12-deficiency than serum cobalamin because it represents the biologically active fraction of cobalamin in humans and may be depleted first in subclinical cobalamin deficiency. HoloTC measurements appear to have slighter better sensitivity; however, the specificity of this assay remains to be determined (Oberley & Yang, 2013). It also is not yet clinically validated or available for widespread use (Langan & Goodbred, 2017).

Criteria	Sensitivity	Specificity	Pitfalls
Serum total cobalamin (<200 pg/mL)	95–97%	Uncertain, possibly <80%	**Elevated levels seen with:** Assay technical failure Occult malignancy Alcoholic liver disease Renal disease *Decreased levels also seen with*: Haptocorrin deficiency Folate deficiency Plasma cell myeloma HIV Pregnancy
Elevated serum methylmalonic acid	>95%	Uncertain	*Elevated levels seen with*: Renal insufficiency Hypovolemia Congenital metabolic defects Amyotrophic lateral sclerosis
Elevated serum homocysteine	>95%	Uncertain, less specific than methylmalonic acid	**Elevated levels seen with:** Folate or pyridoxine deficiency Renal insufficiency Hypovolemia Hypothyroidism Psoriasis Congenital metabolic defects Neurodegenerative disease Malignancy Medications
Decreased serum holotranscobalamin	Similar to total cobalamin	Uncertain	**Levels may be affected by:** Liver disease Macrophage activation Autoantibodies

Clinical Utility and Validity

Health Quality Ontario (HQO) performed an extensive meta-analysis of the clinical utility of B12 testing in patients with suspected dementia or cognitive decline because more than 2.9 million serum B12 tests were performed in Ontario alone in 2010 (HQO, 2013). HQO included data from eighteen different studies to address three questions:

1. "Is there an association between vitamin B12 deficiency and the onset of dementia or cognitive decline?
2. Does treatment with vitamin B12 supplementation improve cognitive function in patients with dementia or cognitive decline and vitamin B12 deficiency?
3. What is the effectiveness of oral versus parenteral vitamin B12 supplementation in those with confirmed vitamin B12 deficiency?"

They concluded that "This evidence-based analysis assessed the usefulness of serum vitamin B12 testing as it relates to brain function. This review found very low-quality evidence that suggests a connection between high plasma homocysteine levels (a by-product of B vitamin metabolism in the body) and the onset of dementia. Moderate quality of evidence indicates treatment with vitamin B12 does not improve brain function. Moderate quality of evidence also indicates treatment using oral vitamin B12 supplements is as effective as injections of vitamin B12" (HQO, 2013).

Another meta-analysis, completed in 2015, utilized data from 12 studies and a total of 34,481 patients to determine if vitamin B12, vitamin B6, and folic acid supplementation affected homocysteine levels and/or reduced the risk of cardiovascular disease (Li, Li, Qi, & Shen, 2015). A combination of vitamin B12, vitamin B6, and folic acid was found to significantly reduce plasma homocysteine levels, but it did not seem to impact cardiovascular disease risk (Li et al., 2015). Therefore, it was concluded that vitamin B12 should not be utilized as a cardiovascular disease prevention method. Additional research has also concluded that the "Use of vitamin B12 in patients with elevated serum homocysteine levels and cardiovascular disease does not reduce the risk of myocardial infarction or stroke, or alter cognitive decline" (Langan & Goodbred, 2017).

In other indications, vitamin B12 has recently been utilized as a biomarker for patients undergoing therapeutic treatment for tuberculosis (TB); vitamin B12 serum concentrations were observed to have significant differences in TB patients between baseline and 6 months after anti-TB treatment (ATT), attributing the decrements in vitamin B12 to the body "reclaiming normal physiological function of the affected organs and immune function improv[ing] by cleaning or a rapid drop in bacterial load" (Gebremicael et al., 2019). Gebremicael et al. (2019) also found that HIV and HAART therapy status of TB patients at baseline had "no effect on the concentration levels of vitamin B12 and vitamin A," and HAART treatment did not affect vitamin B12 serum concentration in ATT treated HIV+/TB+ patients.

Wolffenbuttel, Heiner-Fokkema, Green, and Gans (2020) recently conducted a study obtaining data from the general population of National Health and Nutrition Examination Survey (NHANES). A total of 24462 patients were included. The authors found a positive association between low serum B12 concentration and all-cause mortality (hazard ratio [HR] = 1.39), as well as between low serum B12 concentration and cardiovascular mortality (HR = 1.64). The authors also found a positive association of high serum B12 concentration and cardiovascular mortality (HR = 1.45), although the authors noted that participants with diagnoses such as hyperlipidemia and CVD tended to use vitamin B12-containing supplements more often than those without such diagnoses. However, the authors did not find an association between vitamin B12 supplement intake and mortality. This demonstrates the importance of testing for B12 in the long run to adjust dietary intake and reduce mortality.

V. Guidelines and Recommendations

American Academy of Family Physicians (AAFP)

The AAFP does not recommend screening persons at average risk of vitamin B12 deficiency. Screening should be considered in patients with risk factors, and diagnostic testing should be considered in those with suspected clinical manifestations. These manifestations are listed below:

- "Cutaneous
  - Hyperpigmentation
  - Jaundice
  - Vitiligo
- Gastrointestinal
  - Glossitis
- Hematologic
  - Anemia (macrocytic, megaloblastic)
  - Leukopenia
  - Pancytopenia
  - Thrombocytopenia
  - Thrombocytosis
- Neuropsychiatric
  - Areflexia
  - Cognitive impairment (including dementia-like symptoms and acute psychosis)
  - Gait abnormalities
  - Irritability
  - Loss of proprioception and vibratory sense
  - Olfactory impairment
  - Peripheral neuropathy"

"The recommended laboratory evaluation for patients with suspected vitamin B12 deficiency includes a complete blood count and serum vitamin B12 level. In patients with a normal or low-normal serum vitamin B12 level, complete blood count results demonstrating macrocytosis, or suspected clinical manifestations, a serum methylmalonic acid level is an appropriate next step and is a more direct measure of vitamin B12's physiologic activity; although not clinically validated or available for widespread use, measurement of holotranscobalamin, the metabolically active form of vitamin B12, is an emerging method of detecting deficiency."

AAFP also notes that different causes of vitamin B12 deficiency have corresponding "time to improvement" after initiation of treatment. For abnormalities related to "Homocysteine or methylmalonic acid level, or reticulocyte count", AAFP lists an "expected time until improvement" of one week; for neurologic symptoms; six weeks to three months; for anemia, leukopenia, mean corpuscular volume, or thrombocytopenia; eight weeks.

Finally, AAFP lists risk factors for vitamin B12 deficiency, which are listed below:

- "Decreased ileal absorption
  - Crohn disease

  - Ileal resection
  - Tapeworm infection
- Decreased intrinsic factor
  - Atrophic gastritis
  - Pernicious anemia
  - Postgastrectomy syndrome (includes Roux-en-Y gastric bypass)
- Genetic
  - Transcobalamin II deficiency
- Inadequate intake
  - Alcohol abuse
  - Patients older than 75 years
  - Vegans or strict vegetarians (including exclusively breastfed infants of vegetarian/vegan mothers)
- Prolonged medication use
  - Histamine H2 blocker use for more than 12 months
  - Metformin use for more than four months
  - Proton pump inhibitor use for more than 12 months" (Langan & Goodbred, 2017).

The AAFP comments on pernicious anemia, stating that "Patients diagnosed with vitamin B_{12} deficiency whose history and physical examination do not suggest an obvious dietary or malabsorptive etiology should be tested for pernicious anemia with anti-intrinsic factor antibodies (positive predictive value = 95%), particularly if other autoimmune disorders are present." AAFP also notes that "Patients with pernicious anemia may have hematologic findings consistent with normocytic anemia" (Langan & Goodbred, 2017).

In their "Update on Vitamin B12 Deficiency" published in the *American Family Physician*, Langan and Zawistoski (2011) remarked that "No major medical organizations, including the U.S. Preventive Services Task Force, have published guidelines on screening asymptomatic or low-risk adults for vitamin B12 deficiency, but high-risk patients, such as those with malabsorptive disorders, may warrant screening" (Langan & Zawistoski, 2011).

American College of Gastroenterology (ACG)

According to the ACG, "people with newly diagnosed celiac disease should undergo testing and treatment for micronutrient deficiencies. Deficiencies to be considered for testing should include, but not be limited to, iron, folic acid, vitamin D, and vitamin B12 (conditional recommendation, low level of evidence)" (Rubio-Tapia et al., 2013).

American Academy of Neurology (AAN)

"The American Academy of Neurology recommends serum vitamin B12 testing as part of the assessment of elderly patients with dementia" (Knopman et al., 2001). Currently, this guideline is being updated as of 10/26/2020.

British Committee for Standards in Haematology

"Serum cobalamin currently remains the first-line test, with additional second-line plasma methylmalonic acid to help clarify uncertainties of underlying biochemical/functional deficiencies.

Serum holotranscobalamin has the potential as a first-line test, but an indeterminate 'grey area' may still exist. Plasma homocysteine may be helpful as a second-line test but is less specific than methylmalonic acid. The availability of these second-line tests is currently limited" (Devalia et al., 2014).

The Doctors of BC (formerly the British Columbia Medical Association)

The Doctors of BC recommend vitamin B12 testing for individuals with "unexplained neurologic symptoms such as paresthesias, numbness, poor motor coordination, memory lapses, or cognitive and personality changes," and anemia. They also recommend consideration of testing of elderly individuals (>75 years old), those with inflammatory bowel disease (of small intestine), gastric or small intestine resection, prolonged vegan diet, and long-term use of H2 receptor antagonists or proton pump inhibitors (at least 12 months), or metformin (at least 4 months).

American Association of Clinical Endocrinologists (AACE) and the American College of Endocrinology (ACE) and the Obesity Society (TOS)

"Vitamin B12 levels should be checked periodically in older adults and patients on metformin therapy (Grade A, BEL 1). With the exception of early treatment of patients with neurologic symptoms, pernicious anemia, or malabsorptive bariatric surgery requiring parenteral (intramuscular or subcutaneous) vitamin B12 replacement, patients with vitamin B12 deficiency can generally be treated with oral vitamin B12 (1,000 µg per day of oral crystalline cobalamin) and may benefit from increasing the intake of vitamin B12 in food (Grade A, BEL 1)" (Gonzalez-Campoy et al., 2013).

American Association of Clinical Endocrinologists (AACE), the Obesity Society, and American Society for Metabolic & Bariatric Surgery (ASMBS)

"Baseline and postoperative evaluation for vitamin B12 deficiency is recommended in all bariatric surgery and annually in those with procedures that exclude the lower part of the stomach (e.g., LSG, RYGB) (Grade B; BEL 2)" (Mechanick et al., 2013).

American Society for Metabolic and Bariatric Surgery (ASMBS) Integrated Health Nutritional Guidelines (2016 Update)

Concerning vitamin B12 screening and weight loss surgical (WLS) practices, the ASMBS states that "routine pre-WLS screening of B12 is recommended for all patients (Grade B, BEL 2)." Further, serum MMA [methylmalonic acid] testing is recommended to evaluate a possible B12 deficiency for both asymptomatic and symptomatic patients as well as in "those with history of B12 deficiency or preexisting neuropathy (Grade B, BEL 2)"

The ASMBS also makes the following recommendations for post-WLS nutrient screening:

- "Routine post-WLS screening of vitamin B12 status is recommended for patients who have undergone RYGB [Roux-en-Y gastric bypass], SG [sleeve gastrectomy], or BPD/DS [biliopancreatic diversion/duodenal switch]."
- "More frequent screening (e.g., every 3 mo) is recommended in the first post-WLS year, and then at least annually or as clinically indicated for patients who chronically use medications that exacerbate risk of B12 deficiency: nitrous oxide, neomycin, metformin, colchicine, proton pump inhibitors, and seizure medications."

- "Serum B12 may not be adequate to identify B12 deficiency. It is recommended to include serum MMA with or without homocysteine to identify metabolic deficiency of B12 in symptomatic and asymptomatic patients and in patients with history of B12 deficiency or preexisting neuropathy." (Parrott et al., 2017).

American Association of Clinical Endocrinologists/American Collee of Endocrinology (AACE/ACE), The Obesity Society (TOS), American Society for Metabolic & Bariatric Surgery (ASMBS), Obesity Medicine Association (OMA), and American Society of Anesthesiologists (ASA) (2019 Update)

The AACE/ACE, TOS, ASMBS, OMA, and ASA published clinical practice guidelines for perioperative nutrition, metabolic, and nonsurgical support of patients undergoing bariatric procedures in 2019. In the preprocedure [sic] checklist, the recommendation includes "nutrient screening with iron studies, B12, and folic acid (RBC folate, homocysteine, methylmalonic acid optional), and 25-vitamin D (vitamins A and E optional); consider more extensive testing in patients undergoing malabsorptive procedures based on symptoms and risks. In postprocedure [sic], for early postoperative care, vitamin B12 should be assessed "as needed for normal range levels," and in follow-up "annually; MMA and HCy optional; then q 3-6 mo if supplemented)" (Mechanick et al., 2019). In addition, the societies state:

- Vitamin B12 screening is "recommended for patients who have undergone RYGB [Roux-en-Y gastric bypass], SG [sleeve gastrectomy], or BPD/DS (biliopancreatic diversion/duodenal switch]"
- "Patients who become pregnant following bariatric procedure should have nutritional surveillance and laboratory screening for nutrient deficiencies every trimester, including iron, folate, vitamin B12, vitamin D, and calcium, and if after a malabsorptive procedure, fat-soluble vitamins, zinc, and copper (Grade D)
- Baseline and annual post-bariatric procedure evaluation for vitamin B12 deficiency should be performed in all patients (Grade B; BEL 2)
- More frequent aggressive case finding (e.g., every 3 mo) should be performed in the first postoperative year and then at least annually or as clinically indicated for patients who chronically use medications that exacerbate risk of B12 deficiency, such as nitrous oxide, neomycin, metformin, colchicine, proton-pump inhibitors, and seizure medications (Grade B, BEL 2)
- Because serum B12 may not be adequate to identify B12 deficiency, consider measuring serum methylmalonic acid, with or without homocysteine, to identify a metabolic deficiency of B12 in symptomatic and asymptomatic patients and in patients with a history of B12 deficiency or preexisting neuropathy (Grade B, BEL 2)
- B12 status should be assessed in patients on higher-dose folic acid supplementation (>1000 µg/d) to detect a masked B12- deficiency state (Grade D)" (Mechanick et al., 2019).

British Obesity & Metabolic Surgery Society (BOMSS) (2020 Update)

The BOMSS released 2020 perioperative and postoperative guidelines on biochemical monitoring and micronutrient replacement for patients undergoing bariatric surgery. On measuring vitamin B12 concentrations, the BOMSS has included checking a "full blood count including haemoglobin, ferritin, folate and vitamin B12 levels" in their preoperative nutritional assessment with a grade B and evidence level (EL) of 2. For postoperative care and biochemical monitoring, the BOMSS stated,

- "check vitamin B12 levels at regular intervals following, SG, RYGB and malabsorptive procedures such as BPD/DS (Grade B, EL2)

- Consider the following frequency of vitamin B12 levels: 3, 6 and 12 months in the first year and at least annually thereafter so that changes in status may be detected (GPP – good practice point)"

With relation to folic acid deficiency, O'Kane et al. (2020) mentions, "check and treat for vitamin B12 deficiency, before initiating folic acid treatment to avoid precipitation of subacute combined degeneration of the spinal cord (Grade D, EL4)." For any presence of neurological symptoms/Wernicke's encephalopathy, the guidelines recommend to "check for vitamin B12, copper, and vitamin E deficiencies and treat (GPP)." In pregnant women after undergoing bariatric surgery, checking for vitamin B12 deficiency, among other nutritional deficiencies, has been recommended for each trimester and prior to additional folic acid supplementation in the preconception period (O'Kane et al., 2020).

Guidelines for Diagnosis and Management of the Cobalamin-related Remethylation Disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR Deficiency

This international consortium of scientists from Europe and the U.S. issued guidelines "within the frame of the 'European network and registry for homocystinurias and methylation defects' (E-HOD) project." For Recommendation 5, they state (Quality of the evidence: moderate), "we strongly recommend that in the case of high total homocysteine, plasma and urine samples for determination of MMA, methionine, folate and vitamin B12 are to be obtained before treatment is started" (Huemer et al., 2017).

The American Diabetes Association (ADA)

The ADA states that in patients with type 2 diabetes, the long-term use of metformin may be associated with a vitamin B12 deficiency; therefore, a Grade B recommendation has been made which states that the "periodic measurement of vitamin B12 levels should be considered in metformin-treated patients, especially in those with anemia or peripheral neuropathy" (ADA, 2020a). The ADA also recommended that "measurement of vitamin B12 levels should be considered in patients with type 1 diabetes and peripheral neuropathy or unexplained anemia" as well (ADA, 2020).

American Psychiatric Association (APA)

The APA released guidelines which include a table of the physical complications of anorexia nervosa and potential laboratory testing methods. This table contains a few vitamin assays that may be used to monitor endocrine or metabolic processes including vitamin B12 assays "in severe cases" (Yager et al., 2006).

U.S. Preventative Services Task Force (USPSTF)

Currently, the USPSTF has not published guidelines for vitamin B12 deficiency screenings of asymptomatic or low-risk adults.

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA has cleared numerous devices including needles, reagents, instrumentation, and imaging systems for use in prostate biopsy. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of

1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82607	Cyanocobalamin (Vitamin B-12)
83090	Homocysteine
83921	Organic acid, single, quantitative

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Vitamin D Testing

Policy #: AHS – G2005	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/19/22, 3/13/23 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Vitamin D is a precursor to steroid hormones and plays a key role in calcium absorption and mineral metabolism. Vitamin D promotes enterocyte differentiation and the intestinal absorption of calcium. Other effects include a lesser stimulation of intestinal phosphate absorption, suppression of parathyroid hormone (PTH) release, regulation of osteoblast function, osteoclast activation, and bone resorption (Pazirandeh & Burns, 2021).

Vitamin D is present in nature in two major forms. Ergocalciferol, or vitamin D2, is found in fatty fish (e.g., salmon and tuna) and egg yolks, although very few foods naturally contain significant amounts of vitamin D. Cholecalciferol, or vitamin D3, is synthesized in the skin via exposure to ultraviolet radiation present in sunlight. Some foods are also fortified with vitamin D, most notably milk and cereals (Sahota, 2014).

II. Related Policies

Policy Number	Policy Title
AHS-G2164	Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

Vitamin D Testing, continued

1. For individuals with an underlying disease or condition which is specifically associated with vitamin D deficiency or decreased bone density (see Note 1). Or for individuals suspected of hypervitaminosis of Vitamin D, 25-hydroxyvitamin D serum testing **MEETS COVERAGE CRITERIA.**
2. As part of the total 25-hydroxyvitamin D analysis, testing for D2 and D3 fractions of 25-hydroxyvitamin D **MEETS COVERAGE CRITERIA**.
3. For individuals who have documented vitamin D deficiency, repeat testing for serum 25-hydroxyvitamin D at least 12 weeks after the initiation of vitamin D supplementation therapy **MEETS COVERAGE CRITERIA** with the following restrictions:
    a. Repeat testing for the monitoring of supplementation therapy should not exceed 2 testing instances per year until the therapeutic goal is achieved.
    b. Once therapeutic range has been reached, annual testing, meets coverage criteria.
4. For the evaluation or treatment of conditions that are associated with defects in vitamin D metabolism (see Note 2), 1,25-dihydroxyvitamin D serum testing **MEETS COVERAGE CRITERIA.**
5. The following testing **DOES NOT MEET COVERAGE CRITERIA:**
    a. Measurement of serum 1,25-dihydroxyvitamin D to screen for vitamin D deficiency.
    b. Routine screening for vitamin D deficiency with serum testing in asymptomatic individuals and/or during general encounters

Notes:

Note 1: Indications that support medical necessity for serum measurement of 25-hydroxyvitamin D are as follows:

A. Biliary cirrhosis and other specified disorders of the biliary tract
B. Blind loop syndrome
C. Celiac Disease
D. Coronary artery disease in individuals where risk of disease progression is being considered against benefits of chronic vitamin D and calcium therapy
E. Dermatomyositis
F. Eating disorders
G. Having undergone, or for those who have been scheduled for, bariatric procedures such as Roux-en-Y gastric bypass, sleeve gastrectomy, or biliopancreatic diversion with or without duodenal switch
H. Hypercalcemia, hypocalcemia or other disorders of calcium metabolism
I. Hyperparathyroidism or hypoparathyroidism
J. Individuals receiving hyperalimentation
K. Inflammatory bowel disease (Crohn's disease and ulcerative colitis)
L. Intestinal malabsorption
M. Liver cirrhosis

Vitamin D Testing, continued

N. Long term use of anticonvulsants, glucocorticoids and other medications known to lower vitamin D levels

O. Malnutrition

P. Myalgia and other myositis not specified

Q. Myopathy related to endocrine diseases

R. Neoplastic hematologic disorders

S. Obesity

T. Osteogenesis imperfecta

U. Osteomalacia

V. Osteopetrosis

W. Osteoporosis

X. Pancreatic steatorrhea

Y. Primary or miliary tuberculosis

Z. Psoriasis

AA. Regional enteritis

BB. Renal, ureteral or urinary calculus

CC. Rickets

DD. Sarcoidosis

EE. Stage III-V Chronic Kidney Disease and End Stage Renal Disease

FF. Systemic lupus erythematosus

Note 2: Indications that support medical necessity for serum testing of 1,25-dihydroxyvitamin D are as follows:

A. Disorders of calcium metabolism

B. Familial hypophosphatemia

C. Fanconi syndrome

D. Hyperparathyroidism or hypoparathyroidism

E. Individuals receiving hyperalimentation

F. Neonatal hypocalcemia

G. Osteogenesis imperfecta

H. Osteomalacia

I. Osteopetrosis

J. Primary or miliary tuberculosis

K. Renal, ureteral or urinary calculus

L. Rickets

M. Sarcoidosis

N. Stage III-V Chronic Kidney Disease and End-Stage Renal Disease

IV. Scientific Background

Vitamin D is an important nutrient that helps the body absorb calcium and maintain adequate bone strength. In order to be used in the metabolic process, vitamin D that is consumed or formed in the skin must first be activated via the addition of hydroxyl groups. Two forms of activated vitamin D are found in human circulation: 25-hydroxyvitamin D (calcidiol or 25OHD) and 1,25-dihydroxyvitamin D (calcitriol). 25-hydroxyvitamin D is the predominant and most stable form, but 1,25-dihydroxyvitamin D is the metabolically active form. The initial activation step occurs in the liver, where 25OHD is synthesized, and the second hydroxyl group is added in the kidney, creating the fully activated 1,25-dihdroxy form (Sahota, 2014).

25OHD has a half-life of 15 days in the circulation, whereas 1,25-dihydroxyvitamin D has a much shorter circulating half-life of 15 hours. Consequently, measurement of serum 25OHD is generally accepted as the preferred test to evaluate an individual's vitamin D status despite lack of standardization between methods and laboratories (Glendenning & Inderjeeth, 2012; Sahota, 2014; Scott et al., 2015).

Vitamin D deficiency typically is defined as a serum 25OHD level less than 20 ng/ml, and certain organizations consider <30 ng/ml as insufficient. Trials of vitamin D supplementation (Chapuy et al., 2002; B. Dawson-Hughes, Harris, Krall, & Dallal, 1997; Sanders et al., 2010; Trivedi, Doll, & Khaw, 2003) and the Institute of Medicine (IOM) systematic review (Ross et al., 2011) recommend maintaining the serum 25OHD concentration between 20 and 40 ng/mL (50 to 100 nmol/L), whereas other experts favor maintaining 25OHD levels between 30 and 50 ng/mL (75 to 125 nmol/L). Experts agree that levels lower than 20 ng/mL are suboptimal for skeletal health. The optimal serum 25OHD concentrations for extra-skeletal health have not been established (Dawson-Hughes, 2022). Approximately 15% of the U.S. pediatric population suffers from either vitamin D deficiency or insufficiency. Limited sun exposure and the use of sunscreen compromises production of vitamin D, contributing to low 25OHD levels. "UVB absorption is blocked by artificial sunscreens, and sunscreens with a sun protection factor (SPF) of 30 can decrease vitamin D synthetic capacity by as much as 95 percent" (Madhusmita, 2022). Also, "vitamin D deficiency has been reported in dark-skinned immigrants from warm climates to cold climates in North America and Europe" (Dawson-Hughes, 2021). For example, a study by Awumey and colleagues found that Asian Indians who immigrated to the U.S. were considered vitamin D insufficient or deficient even after the administration of 25OHD. "Thus, Asian Indians residing in the U.S. are at risk for developing vitamin D deficiency, rickets, and osteomalacia" (Awumey, Mitra, Hollis, Kumar, & Bell, 1998).

Vitamin D deficiency has been associated with important short- and long-term health effects, such as rickets, osteomalacia, and the risk of osteoporosis (Sahota, 2014). Rickets in children can result in skeletal deformities. To prevent nutritional rickets in infants, vitamin D supplementation is recommended at 400 IU/day; personalized dosages are possible and would require 25OHD testing (Zittermann et al., 2019). In adults, osteomalacia can result in muscular weakness, bone weakness, and osteoporosis which leads to an increased risk for falls and fractures (Granado-Lorencio et al., 2016).

Vitamin D Testing, continued

A role for vitamin D has been suggested in several other conditions and metabolic processes including, but not limited to, cancer, cardiovascular disease, hypertension, diabetes, and preeclampsia. While vitamin D insufficiency has been associated with several cancer types, inconsistencies cause discrepancies in suggested treatment methods; currently, no official institutional guidelines recommend a dietary vitamin D supplementation for cancer prevention (McNamara & Rosenberger, 2019). 25-hydroxyvitamin D (25OHD) is the accepted biomarker of circulating vitamin D, and in utilization of this biomarker, researchers have reported an association between a high vitamin D production rate and a lowered risk of colorectal cancer (Weinstein et al., 2015). Further, low concentrations of 25OHD have been associated with a high risk of cardiovascular disease and mortality, suggesting that patients deficient in vitamin D have an increased risk in developing cardiovascular disease (Crowe et al., 2019). However, conclusive evidence for the role of vitamin D in these conditions is not available (Aspray et al., 2014; Ross et al., 2011). Based on controversial evidence, researchers continue to emphasize the fact that vitamin D supplementation is not an accepted prevention method for cardiac events or cancer (Ebell, 2019).

Certain other conditions may impact an individual's ability to absorb or activate vitamin D, thereby resulting in vitamin D deficiency. These include, but are not limited to, celiac disease, liver cirrhosis, chronic kidney disease, and bariatric surgery. Since Vitamin D is fat soluble, any impact on fat absorption or storage may have an effect on circulating vitamin D levels (Dawson-Hughes, 2021; Fletcher et al., 2019.

According to the Institute of Medicine (IOM), routine dietary supplementation with vitamin D is recommended for most individuals. While there are no differences with regards to gender and recommended daily dose of vitamin D, there are differences depending on age. The IOM recommends a dietary allowance of 600 IU for individuals up to 70 years old, and 800 IU for individuals older than 70 (Ross et al., 2011), although these recommendations have been met with some criticism as being too low to adequately impact vitamin D levels in some individuals. The USPSTF recommends against daily supplementation with 400 IU or less of vitamin D3 and 1000 mg or less of calcium for the primary prevention of fractures in noninstitutionalized postmenopausal women (V. A. Moyer, 2013).

Vitamin D toxicity is very rare and occurs only when levels of 25OHD are >500 nmol/L [>200 ng/mL], which is well above the level considered sufficient. Vitamin D toxicity may cause hypercalciuria, hypercalcemia, renal stones, and renal calcification with renal failure (V. A. Moyer, 2013). Additional research suggests that excess 25-hydroxyvitamin D3 aggravates tubulointerstitial injury (Kusunoki et al., 2015).

Insource Diagnostics has developed two similar quantitative laboratory developed tests (LDTs) termed Sensieva Vena™ 25OH Vitamin D2/D3 and Droplet 25OH Vitamin D2/D3 (InSourceDx, 2019a, 2019b). These assays utilize liquid chromatography coupled with mass spectrometry (LC/MS/MS) to measure both D2 and D3. The LC/MS/MS assessment technique is the apparent gold standard for vitamin D2 and D3 measurement, and is the only currently available method to measure both vitamins individually (InSourceDx, 2019b). These assays may assist in the measurement of several ailments related to abnormal vitamin D levels including parathyroid function, dietary absorption, calcium metabolism, and vitamin D treatment effectiveness; serum, plasma and blood microsamples can be utilized for these tests (InSourceDx, 2019a). The 20uL serum/plasma method of the Sensieva™ 25OH Vitamin D2/D3 LDT was approved by the CDC's VDSCP in 2017-2018 (CDC, 2019). This test is no longer certified by the CDC's VDSCP and as of May 2020 Insource Diagnostics website has been removed. Therefore, it is unclear if this test is still available.

Analytical Validity

Serum or plasma concentration of 25OHD can be measured using a number of assays, including ELISA, between D2 and D3. These methods "can individually quantitate and report both analytes, in addition to providing a total 25-hydroxyvitamin D concentration" (Krasowski, 2011). RIA-based assays for 25OHD can have intra- and inter-assay variations of 8 – 15%, and the Immunodiagnostic Systems (IDS)-developed RIA has a reported 100% specificity for D3 and 75% for D2 (Holick, 2009). "For most HPLC and LC-MS/MS methods extraction and procedural losses are corrected for by the inclusion of an internal standard which, in part, may account for higher results compared to immunoassay" (Wallace, Gibson, de la Hunty, Lamberg-Allardt, & Ashwell, 2010). Even though LC-MS/MS is considered to be the gold standard of measuring 25OHD and its metabolites, only approximately 20% of labs report using it (Avenell, Bolland, & Grey, 2018). One study reports that 46% of samples measured using LC-MS/MS were classified as vitamin D-deficient whereas, when the samples were measured using an immunoassay method, 69% were vitamin D-deficient (<30 nmol/L) (Annema, Nowak, von Eckardstein, & Saleh, 2018).

The Centers for Disease Control and Prevention (CDC) have developed a vitamin D standardization certification program (VDSCP). This program helps to ensure that all LDT vitamin D tests are accurate and reliable by evaluating the performance and overall reliability of these assessments over time, supplying reference measurements for both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, and providing technical support to additional programs and studies (CDC, 2017b).

Due to the great variability among the different assays used to measure vitamin D levels, the VDSCP was created. Interassay variability yields an inadequate basis to establish if 25OHD increases or decreases the risk of non-skeletal diseases and hampers the development of evidence-based guidelines and policies (Sempos & Binkley, 2020). VDSCP studies can either be retrospective or prospective; therefore, standardization of national nutrition survey data may be performed. For example, it was originally thought, based on reports from the National Health and Nutrition Examination Surveys (NHANES), that there had been a dramatic decline in mean 25OHD levels in the US population from 1990 to the period 2001–2004. DiaSorin Radioimmunoassay was used to measure 25OHD levels in these surveys. However, after standardizing the results using VDSCP methods, it was found that the mean 25OHD levels were stable from 1990-2004 (Sempos et al., 2018). The VDSCP program established four steps to achieve standardization, as described by:

1. "Fit for use…means that assay chosen will perform appropriately and provide standardised measurements in the patient/study populations in the conditions for which it will be used…[as] some immunoassays do not function appropriately in all patient populations.
2. [Assay is] Certified by the CDC Vitamin D Standardization Certification Program as being standardised and having an appropriate measurement range or be a documented standardised laboratory-developed HPLC or LC-MS/MS assay with an appropriate measurement range…see which ones are currently, or have been in the past, certified by the CDC as meeting VDSP performance criteria of having a total (coefficient of variation) CV≤10% and a mean bias with the range of –5 to +5%... VDSP recommends using an assay that does have an appropriate measurement range for the population it will be used in; for example, it should be able to measure 25(OH)D in persons who are deficient.
3. Appropriate level of assay precision and accuracy…it has been recommended that a standardised LC-MS/MS assay be selected.

4. [The assay] Meets VDSP assay standardisation criteria in your 'hands' or laboratory…. We recommend a testing period in order to verify that an immunoassay is standardized especially since there is generally very little an individual laboratory can do to 'calibrate' an immunoassay" (Sempos & Binkley, 2020).

Clinical Utility and Validity

A retrospective study of 32,363 tests of serum 25OHD found that a significant proportion of the lab requests were unjustified by medical criteria, and "that clinical and biochemical criteria may be necessary to justify vitamin D testing but not sufficient to indicate the presence of vitamin D deficiency" (Granado-Lorencio et al., 2016).

The table below lists the criteria used for vitamin D testing in the study by Granado and colleagues. (Granado-Lorencio et al., 2016).

Clinical conditions
– Differential diagnosis (i.e. hypercalcemia)
– Undernourished subjects
– Malabsorption syndromes (i.e. celiac disease, Chron's disease, radiation enteritis)
– Eating disorders (i.e. morbid obesity, anorexia and bulimia)
– Candidates for bariatric surgery
– Conditions associated with altered calcium, phosphorus or vitamin D metabolism (i.e. osteoporosis, rickets, renal disease, liver failure, multiple mieloma, sarcoidosis, hyper/hypoparathyroidism, liver and kidney transplants)
– Diseases related to low or null sun exposure (i.e. lupus, porphyria)
– Vitamin D-related inborn errors of metabolism
Therapeutic criteria
– Pharmacotherapy associated with increased vitamin D catabolism (i.e. antiseizure drugs, glucocorticoids)
– Treatment for AIDS and tuberculosis
– Monitorization of vitamin D treatment
Biochemical indicators
– Alterations of serum or urine levels of calcium and phosphorus
– Elevation of alkaline phosphatase (in the absence of altered liver enzymes or growth)
– Serum levels of parathyroid hormone out of the reference range (14–72 pg/mL)
– Previous (<6 months) serum values of 25-OH-vitamin D out of the reference interval (<37.5 or >160 nmol/L)

A meta-analysis study by Bolland and colleagues of 81 randomized controlled trials with a combined total of 53,537 participants measured the effects, if any, vitamin D supplementation had on fractures, falls, and bone density. They found that there was no clinically relevant difference in bone mineral density at any site between the control and experimental groups; moreover, "for total fracture and falls, the effect estimate lay within the futility boundary for relative risks of 15%, 10%, 7.5%, and 5% (total fracture only), suggesting that vitamin D supplementation does not reduce fractures or falls by these amounts. Our findings suggest that vitamin D supplementation does not prevent fractures or falls or clinically meaningful effects on bone mineral density. There were no differences between the effects of higher and lower doses of vitamin D. There is little justification to use vitamin D supplements to maintain or improve musculoskeletal health. This conclusion should be reflected in clinical guidelines" (Bolland et al., 2018).

A prospective study by Hao and colleagues aims to determine whether 25OHD levels is associated with mortality or the ability to walk in a patient cohort after hip fracture surgery. Each year, 300,000 elderly patients, 75% who are women, are hospitalized for hip fractures (CDC, 2017a). In this study, 290 elderly patients with hip fractures were included, in which patients with 25OHD deficiency (<12 ng/ml) were

used as the reference group. They observed a 56–64% increased rate of walking in patients who had 25OHD levels > 12 ng/ml at 30 days and 60 days after hip fracture surgery compared with 35% for patients able to walk 30 days postoperatively who had 25OHD levels < 12 ng/ml (Hao, Carson, Schlussel, Noveck, & Shapses, 2020) . It is important to note that only the preoperative 25OHD levels accurately reflect the patient's ability to walk after 30 days, and the postoperative vitamin D status is not related and should not be used to determine clinical or nutritional interventions. Holick (2020) releases a call for action, discussing the data collected by Hao, to establish guidelines which will assess vitamin D status as needed for patients with hip fracture. Holick suggests that "patients aged ≥50 y presenting with fractures, especially those with hip fracture, should be evaluated at intake for their vitamin D status. Consideration should be made to provide vitamin D supplementation if dietary/supplemental intake or blood concentrations of 25(OH)D suggest deficiency" (Holick, 2020).

Another randomized clinical trial administered a monthly high dose of vitamin D to 5,108 participants in order to determine if a relationship exists between increased vitamin D levels and cardiovascular disease prevention. This double-blind trial was placebo-controlled; participants were given an initial dose of 200,000 IU of vitamin D, and then each month after for a range of 2.5-4.2 years were given 100,000 IU of vitamin D (Scragg et al., 2017). Results showed that in a random sample of 438 participants, cardiovascular disease occurred in 11.8% of patients who received vitamin D supplements and in 11.5% of patients who received placebos. This suggests that vitamin D administration does not prevent cardiovascular disease and should not be used for this purpose (Scragg et al., 2017).

Regarding pregnancy, vitamin D deficiency is common around the world and threatens fetal health and growth. Results from 203 Indonesian women who were followed from their first trimester of pregnancy until delivery showed astronomical vitamin D deficiency rates at approximately 75% (Yuniati et al., 2019). Data collected from these women included maternal demography, bloodwork to test ferritin levels, 25(OH) vitamin D results in their first trimester, and the final birthweight of the child after delivery. Final results did not show any association between ferritin, hemoglobin level, and vitamin D in either the first trimester of pregnancy or in the final birthweight of the neonates after delivery; however, the authors suggest that other unknown variables may be important and that nutritional supplementation during pregnancy is still vital (Yuniati et al., 2019).

Research has also been conducted on the association of 25(OH)D levels and SARS-CoV-2 infection. Ribeiro et al. (2021) conducted a retrospective cohort study on 1638 patients tested for SARS-CoV-2 infection and found that "previous insufficient 25(OH)D (<30ng/mL) concentration and high total cholesterol were associated with SARS-CoV-2 infection among adults >48 y in the study population." This may be attributable to the role that vitamin D serves in the immune system and its anti-viral activity through autophagy, as well as its high expression in cells of the lungs, thus rendering those with lower levels of 25(OH)D more susceptible to infection without these defenses (Ul Afshan et al., 2021).

Szerszeń et al. (2022) also investigated the possible correlation between the immunomodulatory effect of vitamin D and the incidence and progression of COVID. From a sample of 505 patients, they quantified serum 25-OHD and analyzed each patient's COVID severity through the serum Vitamin Modified Early Warning Score (MEWS), "which includes respiratory rate, systolic blood pressure, heart rate, temperature and state of consciousness," along with the days spent in the intensive care unit. The results demonstrated that there was no difference in 25-OHD concentration between those with and without COVID as determined by PCR and no correlation "between serum 25-OHD in the COVID(+) group and the need for and time spend in the ICU as well as the MEWS score." However, "multivariate analyses showed a positive

correlation between need for oxygen therapy and lower 25-OHD concentration, as well as older age (P<0.001) and similar positive correlation between need for ventilation therapy with lower 25-OHD concentration, as well as older age (P=0.005)." This signifies the evolving role of vitamin D in and how low serum levels may aid in predicting more complicated treatment courses.

On the other hand, Javed et al. (2020) found that "high serum levels of vitamin D are associated with a lower risk of incidence and progression of [colorectal cancer]." This could make vitamin D testing crucial to identify possible future therapeutic modalities for patients with both low serum vitamin D and colorectal cancer. Like its mechanisms that hinder SARS-CoV-2 infection, such as being pro-apoptotic and anti-inflammatory, vitamin D has been shown to "decrease growth and differentiation of colon epithelial cells." With more large-scale human trials, testing and treatment using vitamin D can become more widely applicable.

It is also known that decreased vitamin D levels are associated with inflammatory bowel disease (IBD), though the mechanisms have not been fully elucidated(Nielsen et al., 2019; Vernia et al., 2022). Studies further suggest that vitamin D supplementation may positively impact the course of IBD, highlighting the utility of vitamin D testing in this patient population. It has been suggested that a daily dose of 2000 IU correlates with improvements in IBD symptoms and patient quality of life (El Amrousy et al., 2021; Goulart & Barbalho, 2022)

V. Guidelines and Recommendations

The Endocrine Society

The Endocrine Society recommends serum testing of 25-hydroxyvitamin D for evaluation of vitamin D status in individuals who are at risk of deficiency, including those with osteoporosis, obesity, or a history of falls. 1,25-dihydroxyvitamin D testing is not recommended for screening of at-risk individuals, due to its very short half-life in circulation, but is recommended for a few conditions in which formation of the 1,25-dihdroxy form may be impaired (Holick et al., 2011).

Institute of Medicine (IOM)

After an extensive evaluation of published studies and testimony from investigators, the Institute of Medicine determined that supplementation with vitamin D is appropriate; however, guidelines regarding the use of serum markers of vitamin D status for medical management of individual patients and for screening were beyond the scope of the Committee's charge, and evidence-based consensus guidelines are not available (Ross et al., 2011).

The Royal Osteoporosis Society (ROS) (formerly known as the National Osteoporosis Society)

The ROS recommends the measurement of serum 25 (OH) vitamin D (25OHD) to estimate vitamin D status in the following clinical scenarios: bone diseases that may be improved with vitamin D treatment; bone diseases, prior to specific treatment where correcting vitamin D deficiency is appropriate; musculoskeletal symptoms that could be attributed to vitamin D deficiency. The guideline also states that routine vitamin D testing is unnecessary where vitamin D supplementation with an oral antiresorptive treatment is already planned and sets the following serum 25OHD thresholds: <30 nmol/l is deficient; 30-50 nmol/l may be inadequate in some people; >50 nmol/l is sufficient for almost the whole population (Aspray et al., 2014).

American College of Obstetricians and Gynecologists (ACOG) Gynecologic Care for Adolescents and Young Women with Eating Disorders

ACOG has stated that in patients with low bone mineral density (BMD), "appropriate calcium (1,000–1,300 mg per day) and vitamin D (600 international units/day) intake can be recommended; however, there is no evidence that vitamin supplementation improves BMD. A patient's 25-hydroxy vitamin D level should be checked and, if less than 30 ng per mL, the patient should be given supplementation for 6–8 weeks in the form of 2,000 international units daily or 50,000 international units weekly (Wassenaar et al., 2018)." Reaffirmed in 2021.

United States Preventive Services Task Force (USPSTF)

The USPSTF recently issued the guideline *Vitamin D, Calcium, or Combined Supplementation for the Primary Prevention of Fractures in Community-Dwelling Adults*, which recommends the following:

"The USPSTF concludes that the current evidence is insufficient to assess the balance of the benefits and harms of vitamin D and calcium supplementation, alone or combined, for the primary prevention of fractures in community-dwelling, asymptomatic men and premenopausal women. (I statement) The USPSTF concludes that the current evidence is insufficient to assess the balance of the benefits and harms of daily supplementation with doses greater than 400 IU of vitamin D and greater than 1000 mg of calcium for the primary prevention of fractures in community-dwelling, postmenopausal women. (I statement) The USPSTF recommends against daily supplementation with 400 IU or less of vitamin D and 1000 mg or less of calcium for the primary prevention of fractures in community-dwelling, postmenopausal women. (D recommendation) These recommendations do not apply to persons with a history of osteoporotic fractures, increased risk for falls, or a diagnosis of osteoporosis or vitamin D deficiency" (USPSTF, 2018).

In the 2022 update to the USPSTF recommendation concerning the use of vitamins for the primary prevention of cardiovascular disease and cancer, they concluded that there was insufficient evidence to assess the efficacy of multivitamins, including those containing vitamin D, in the prevention of cardiovascular disease or cancer (I statement) (O'Connor et al., 2022).

The USPSTF published their recommendation concerning screening of vitamin D deficiency in asymptomatic community-dwelling, nonpregnant adults in 2015. "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults" (I statement) (LeFevre, 2015). It should be noted that this guideline is currently undergoing review in 2019.

American Association of Clinical Endocrinologists, The Obesity Society, and American Society for Metabolic and Bariatric Surgery

"Baseline and annual postoperative evaluation for vitamin D deficiency is recommended after Rouxen-Y gastric bypass (RYGB), sleeve gastrectomy, or biliopancreatic diversion without/with duodenal switch (BPD/ DS)" (Jeffrey I. Mechanick et al., 2019). Minimal daily nutritional supplementation for patients with Rouxen-Y gastric bypass (RYGB) and Lennox-Gastaut syndrome (LSG) all in chewable form initially should be at least 3000 international units of vitamin D (titrated to therapeutic 25-hydroxyvitamin D levels >30 ng/ml). Minimal daily nutritional supplementation for patients with *Laparoscopic adjustable gastric banding* (LAGB) should include at least 3000 international units of vitamin D (titrated to therapeutic 25-dihydroxyvitamin D levels). Patients with severe vitamin D

malabsorption are recommended initial oral doses of vitamin D2 (50,000 IU 1 to 3 times/weekly) or D3 (minimum of 3,000 IU/day to 6,000 IU/day) (Jeffrey I. Mechanick et al., 2019).

American Academy of Pediatrics (AAP)

"Evidence is insufficient to recommend universal screening for vitamin D deficiency… In the absence of evidence supporting the role of screening healthy individuals at risk for vitamin D deficiency in reducing fracture risk and the potential costs involved, the present AAP report advises screening for vitamin D deficiency only in children and adolescents with conditions associated with reduced bone mass and/or recurrent low-impact fractures. More evidence is needed before recommendations can be made regarding screening of healthy black and Hispanic children or children with obesity. The recommended screening is measuring serum 25-OH-D concentration, and it is important to be sure this test is chosen instead of measurement of the 1,25-OH_2-D concentration, which has little, if any, predictive value related to bone health" (Golden & Abrams, 2014).

American College of Obstetricians and Gynecologists (ACOG)

"At this time, there is insufficient evidence to support a recommendation for screening all pregnant women for vitamin D deficiency. For pregnant women thought to be at increased risk of vitamin D deficiency, maternal serum 25-hydroxyvitamin D levels can be considered and should be interpreted in the context of the individual clinical circumstance" (ACOG, 2011). Reaffirmed in 2021.

VI. Applicable State and Federal Regulations

A search of the FDA Device database on 10/12/2020 for "vitamin D" yielded 41 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82306	Vitamin D; 25 hydroxy
82652	Vitamin D; 1, 25 dihydroxy, includes fraction(s), if performed
0038U	Vitamin D, 25 hydroxy D2 and D3, by LC-MS/MS, serum microsample, quantitative Proprietary test: Sensieva™ Droplet 25OH Vitamin D2/D3 Microvolume LC/MS Assay Lab/Manufacturer: InSource Diagnostics

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
8/19/22	Modified coverage criteria #5a and added Guideline #1-K.
3/13/23	Reformatted and modified wording in overall criteria, and added Note #1G to coverage criteria.

G2005 Vitamin D Testing

Vitamin D Testing

Policy #: AHS – G2005	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/19/22, 3/13/23 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Vitamin D is a precursor to steroid hormones and plays a key role in calcium absorption and mineral metabolism. Vitamin D promotes enterocyte differentiation and the intestinal absorption of calcium. Other effects include a lesser stimulation of intestinal phosphate absorption, suppression of parathyroid hormone (PTH) release, regulation of osteoblast function, osteoclast activation, and bone resorption (Pazirandeh & Burns, 2021).

Vitamin D is present in nature in two major forms. Ergocalciferol, or vitamin D2, is found in fatty fish (e.g., salmon and tuna) and egg yolks, although very few foods naturally contain significant amounts of vitamin D. Cholecalciferol, or vitamin D3, is synthesized in the skin via exposure to ultraviolet radiation present in sunlight. Some foods are also fortified with vitamin D, most notably milk and cereals (Sahota, 2014).

II. Related Policies

Policy Number	Policy Title
AHS-G2164	Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

ZIKA Virus Risk Assessment

Policy #: AHS – G2133	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/27/22 (see Section IX)

Disclaimer:

1. Policies are subject to change without notice.
2. Policies outline coverage determinations for SelectHealth Commercial, SelectHealth Advantage® (Medicare/CMS) and SelectHealth Community Care® (Medicaid/CHIP) plans. Refer to the "Policy" section for more information.

I. Policy Description

Zika virus is a flavivirus, closely related to dengue. It is transmitted to humans primarily through the bite of certain infected Aedes genus mosquitoes, and less frequently, via sexual intercourse or blood transfusion (Basu & Tumban, 2016). There is no vaccine or specific medicine for Zika virus (CDC, 2016).

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening
AHS-G2158	Testing for Mosquito- or Tick-Related Infections

III. Indications and/or Limitations of Coverage

Application of coverage criteria depends on an individual's benefit coverage at the time of the request.

For SelectHealth Advantage (Medicare/CMS), coverage is determined by the Centers for Medicare and Medicaid Services (CMS). If a coverage determination has not been adopted by CMS and InterQual criteria are not available, the SelectHealth Commercial policy applies. For the most up-to-date Medicare policies and coverage, please visit their search website or the manual website,

For SelectHealth Community Care (Medicaid), coverage is determined by the State of Utah Medicaid program. If Utah State Medicaid has no published coverage position and InterQual criteria are not available, the SelectHealth Commercial criteria will apply. For the most up-to-date Medicaid policies and coverage, please visit their website or the Utah Medicaid Code Look-Up tool.

1. Zika virus urine, serum, and CSF RNA NAT testing and IgM testing in infants **MEETS COVERAGE CRITERIA** in the following situations:
   a. infants with clinical findings consistent with congenital Zika syndrome and possible maternal Zika virus exposure during pregnancy, regardless of maternal testing results

b. infants without clinical findings consistent with congenital Zika syndrome born to mothers with laboratory evidence of possible Zika virus infection during pregnancy

2. Zika virus NAAT testing of maternal serum and urine specimens **MEETS COVERAGE CRITERIA** in the following situations:
    a. pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus
    b. symptomatic pregnant individuals who have had exposure to Zika virus during pregnancy
    c. asymptomatic pregnant individuals who have had exposure to Zika virus via travel to an area of risk outside of U.S. territories for a time limit of up to 12 weeks post-travel
3. In pregnant individuals with possible exposure to Zika virus AND who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection, Zika virus IgM testing of maternal serum **MEETS COVERAGE CRITERIA**.
4. Zika virus NAAT testing of amniocentesis, placental and fetal tissues **MEETS COVERAGE CRITERIA** in pregnant women with possible exposure to Zika virus and who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection and undergoing amniocentesis.
5. For preconception screening, Zika virus urine and serum NAAT testing and Zika virus serum IgM testing **DOES NOT MEET COVERAGE CRITERIA**.
6. In all non-pregnant individuals presenting ≥14 days after symptoms onset, Zika virus urine and serum NAAT testing and Zika virus serum IgM testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

7. All other tests for diagnosing Zika virus not mentioned above in all other situations and testing of samples other than serum, urine, CSF, amniocentesis, placental and fetal tissues at this time **DO NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Zika virus is a mosquito-borne illness discovered in Uganda in 1947 but has since spread across Asia and to the Americas. Zika infection has been tied to several birth defects. The first human cases of Zika were detected in 1952. Prior to 2007, at least 14 cases of Zika had been documented. Symptoms of Zika are similar to those of many other diseases; therefore, many cases may not have been recognized (CDC, 2016). In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infection in Brazil. On February 1, 2016, the World Health Organization (WHO) declared Zika virus a Public Health Emergency of International Concern (PHEIC) (WHO, 2016d).

The most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis (CDC, 2016). The illness is usually mild with symptoms beginning 2-7 days after being bitten by an infected mosquito, and lasting for several days to a week. Most individuals infected with Zika virus are unaware of the infection, as only a maximum of 25% of people infected will exhibit symptoms. (CDC, 2016; LeBeaud, 2021).

Diagnosis of the Zika virus is definitively established through reverse-transcription polymerase chain reaction (RT-PCR) for Zika virus RNA in all symptomatic patients. Asymptomatic patients are typically not tested aside from pregnant women (LeBeaud, 2021).

Zika virus infection during pregnancy can cause serious birth defects, as the virus may be passed to the developing fetus (CDC, 2016). Moore et al. (2017) published a report detailing characteristic birth defects of Zika-affected children, that included: "(1) severe microcephaly with partially collapsed skull; (2) thin cerebral cortices with subcortical calcifications; (3) macular scarring and focal pigmentary retinal mottling; (4) congenital contractures; and (5) marked early hypertonia and symptoms of extrapyramidal involvement." Other birth defects such as seizures, hearing loss, or cardiac anomalies may also be present. As with adults, a congenital Zika virus infection is confirmed by the presence of Zika RNA in infant serum, urine, or cerebrospinal fluid (Nielsen-Saines, 2019).

Analytical Validity

A diagnosis of Zika is definitively established by real-time RT-PCR (rRT-PCR), which detects Zika virus RNA in serum, urine, or whole blood. Serological testing (detection of the IgM antibody in serum) may also be performed. Plaque reduction neutralization test (PRNT, a specific antibody test for flaviviruses) may be used to confirm an infection if previous tests are inconclusive (LeBeaud, 2021; Petersen, 2018). Several proprietary tests for the assessment of Zika are available directly to consumers. For example, MaterNova (based in Rhode Island) has a "rapid, visual, qualitative immunochromatographic in-vitro assay for the differential detection of IgG & IgM antibodies to Zika virus in human serum, plasma, and/or whole blood samples" (Maternova, 2019). Co-Diagnostics Inc. (based in Salt Lake City) also has a rRT-PCR available for detection of Zika. This test was evaluated at a sensitivity of 98.84% and specificity of 100% (Co-Diagnostics, 2018).

Li et al. (2019) have developed an enzyme-linked immunospot assay performed in a 96-well format for the rapid detection of Zika virus. A monoclonal antibody (11C11) that is known to have a high reactivity and affinity to the Zika virus was used for detection purposes. The authors state that: "Overall, we successfully developed an efficient neutralization test for ZIKV [zika virus] that is high-throughput and rapid (Li et al., 2019)." It has been noted by Ricotta et al. (2019) that antibody-based detection systems are less than ideal due to potential false positive results.

Another testing method has been developed by Ricotta et al. (2019) that uses a chip-based potentiometric sensor and 3D surface molecular imprinting. This sensor system "was able to detect 10^{-1} PFU mL^{-1} ZIKV [zika virus] in a buffered solution under 20 minutes without any sample manipulation" and showed no signs of cross-reactivity in this study (Ricotta et al., 2019). The authors claim that this testing method exhibited high sensitivity and selectivity and could even determine the chirality of amino acids in the sample. However, sensitivity values are not given.

Granger and Theel (2019) have published an evaluation of two enzyme-linked immunosorbent assays and a rapid immunochromatographic assay for the detection of IgM antibodies to Zika virus. This article states that five serological assays have been approved by the FDA in an emergency use situation and include the Chembio DPP Zika IgM system (a rapid immunochromatographic assay), the InBios ZIKV Detect 2.0 IgM antibody capture enzyme-linked immunosorbent assay, and the InBios ZIKV Detect MAC-

ELISA. These three serologic assays were evaluated, using 72 samples, based on the identification of neutralizing antibodies to Zika virus, dengue virus or West Nile virus. "The Chembio DPP Zika ICA and InBios ZIKV 2.0 MAC-ELISA showed 95% specificity in 22 ZIKV/DENV-seronegative specimens and in 13 samples positive for NAbs to non-ZIKV flaviviruses. Comparatively, the InBios ZIKV MAC-ELISA was "presumptive" or "possible Zika positive" in 8 of 12 WNV or DENV PRNT-positive samples and in 12 of 22 PRNT-seronegative sera (Granger & Theel, 2019)." The authors conclude that by replacing the InBios ZIKV MAC-ELISA with the InBios ZIKV 2.0 MAC-ELISA, testing burden will be minimized on laboratories performing PRNT for the identification of neutralizing antibodies.

Clinical Utility and Validity

Reynolds et al. (2017) examined the 2016 United States Pregnancy Registry to estimate the proportion of birth defects of pregnant women exposed to Zika, and out of 972 pregnancies with laboratory evidence of a possible Zika infection, 51 had birth defects (5%). Of the 250 confirmed infections, 24 had birth defects. Similarly, Shiu et al. (2018) evaluated the screening results of the Zika virus in Miami-Dade County in Florida. Of 2327 women screened for Zika, 86 had laboratory evidence of infection, and 2 had congenital Zika "syndrome" (Zika-caused birth defects) (Shiu et al., 2018).

St George et al. (2017) assessed the accuracy of several diagnostic tests for the Zika virus. The authors examined the first 80 Zika-positive patients of New York State with a variety of tests. The Zika virus RNA was detected in urine from 50 patients, serum from 19 patients, and in both media in 11 patients. Average viral loads were found to be larger in the urine sample. Two separate RT-PCR targets were used: one targeted the viral envelope, and the other targeted the *NS2B* genes. Out of the 93 positive samples (from the patients), 41 were positive on both PCRs, 52 were positive on RT-PCR targeting the *NS2B* genes of the virus only, and zero were positive on the RT-PCR that only targeted the viral envelope (St George et al., 2017).

Granger et al. (2017) compared the performance of three enzyme-linked immunosorbent assays (ELISAs) for the assessment of Zika. The three ELISAs compared were the CDC variant, the InBios variant, and the EuroImmun variant. The CDC and InBios were found to compare favorably ("positive agreement, negative agreement, and interrater kappa values ranging from 87.5% to 93.1%, 95.7% to 98.5%, and 0.52 to 0.83, respectively"), but comparison of the EuroImmun ELISA to either CDC or InBios resulted in "positive agreement, negative agreement, and interrater kappa values ranging from 17.9% to 42.9%, 91.7% to 98.6%, and 0.10 to 0.39, respectively." The authors concluded that these assays needed more improvement (Granger et al., 2017).

Lin et al. (2018) discussed the difficulties of interpretation and management based on Zika virus test results. The researchers note that there is no singular testing approach or one test with superior validity, and that tests tend to vary substantially on "(1) what the test seeks to detect; (2) the test's sensitivity and specificity under idealized conditions; and (3) moderators that affect test validity under real world conditions, such as pregnancy status, the timing of a test, what fluids are tested, and cross-reactivity with other, similar viruses." The current tests are very specific, but vary more widely in sensitivity, with "limits of detection ranging across 3 orders of magnitude" (Lin et al., 2018).

Kim et al. (2018) had also developed a rapid diagnostic test (RDT) for detecting IgG/IgM antibodies against Zika virus using "monoclonal antibodies to the envelope (E) and non-structural protein (NS1)." The diagnostic accuracy of this kit was "fairly high; sensitivity and specificity for IgG was 99.0 and 99.3%, respectively, while for IgM it was 96.7 and 98.7%, respectively." However, there were cross reactions with the dengue virus evaluated using anti-Dengue Mixed Titer Performance Panel (PVD201), "in which the Zika RDT showed cross-reactions with [dengue virus] in 16.7% and 5.6% in IgG and IgM, respectively." This research could potentially enable the rapid diagnostic test to be preferable to the traditional RT-PCR in endemic areas (Kim et al., 2018).

Voermans et al. (2019) have published an article regarding the benefits of whole blood samples versus plasma samples in the identification of Zika virus infections. Quantitative RT-PCR was used on whole blood and plasma paired samples taken from 249 patients (227 patients had a suspected Zika virus infection). The authors state, "Our overall results indicate that, in our routine diagnostic algorithm in the absence of whole-blood testing, the infections of 5 of 227 patients would have been identified as probable Zika virus cases, whereas with whole-blood testing, they would have been identified as confirmed cases on the basis of positive qRT-PCR results (Voermans et al., 2019)." Based on these results, the authors implemented whole-blood RT-PCR testing as a routine diagnostic setup for their clinic rather than plasma sample testing.

V. Guidelines and Recommendations

Centers for Disease Control and Prevention

Zika Virus Laboratory Testing

The definitive laboratory diagnosis of Zika virus requires multiple assays and sample types. There are several types of Zika Virus tests available such as RNA NAT (nucleic acid testing), Trioplex Real-time RT-PCR Assay, Serologic test for Zika Virus, Zika MAC-ELISA and Plaque Reduction Neutralization Test (PRNT). They all have their limitations and are recommended or not recommended to use depending on the population being tested (CDC, 2019b).

"Laboratory testing for Zika virus has a number of limitations. Zika virus RNA is only transiently present in body fluids; thus, negative nucleic acid testing (NAT) does not rule out infection. Serologic testing is affected by timing of sample collection: a negative immunoglobulin M (IgM) serologic test result does not rule out infection because the serum specimen might have been collected before the development of IgM antibodies, or after these antibodies have waned. Conversely, IgM antibodies might be detectable for months after the initial infection; for pregnant women, this can make it difficult to determine if infection occurred before or during a current pregnancy. In addition, cross-reactivity of the Zika virus IgM antibody tests with other flaviviruses can result in a false-positive test result, especially in persons previously infected with or vaccinated against a related flavivirus, further complicating interpretation. Limitations of Zika virus IgM antibody assays that were approved under an Emergency Use Authorization have been recognized; both false-positive and false-negative test results have occurred (CDC, 2017c)."

Updated Guidance for Testing of Symptomatic Pregnant Women with Possible Zika Virus Exposure

"Given the decreasing prevalence of Zika virus infection cases in the Americas and emerging data regarding Zika virus laboratory testing, on July 24, 2017, CDC published updated guidance for testing of pregnant women with possible Zika virus exposure. Zika virus NAT testing should be offered as part of routine obstetric care to asymptomatic pregnant women with ongoing possible Zika virus exposure (residing in or frequently traveling to an area with risk for Zika virus transmission); serologic testing is no longer routinely recommended because of the limitations of IgM tests, specifically the potential persistence of IgM antibodies from an infection before conception and the potential for false-positive results. Zika virus testing is not routinely recommended for asymptomatic pregnant women who have possible recent, but not ongoing, Zika virus exposure; however, guidance might vary among jurisdictions (CDC, 2017a)."

Updated Testing Guidance Recommendations

The CDC has published updated guidelines for the testing of Zika virus. The guidelines state that asymptomatic, non-pregnant patients should **not** be tested for Zika virus, and symptomatic non-pregnant patients are **not** recommended to be tested for Zika "based on the current epidemiology of these viruses" (CDC, 2019a). Regarding pregnant women, the CDC states that these women should not travel to areas of known Zika outbreaks. Further, for asymptomatic pregnant women, the following recommendations were given:

- "For asymptomatic pregnant persons living in or with recent travel to the U.S. and its territories, routine Zika virus testing is NOT currently recommended.
- For asymptomatic pregnant women with recent travel to an area with risk of Zika (purple areas) outside the U.S. and its territories, Zika virus testing is NOT routinely recommended, but NAAT testing may still be considered up to 12 weeks after travel.
- Zika virus serologic testing is NOT recommended for asymptomatic pregnant women.
    - Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.
    - There is notable cross-reactivity between dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive (CDC, 2019a)."

For symptomatic pregnant women, the following recommendations were given:

- "For symptomatic pregnant women who had recent travel to areas with active dengue transmission and a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset.
    - The following diagnostic testing should be performed at the same time:
        - Dengue and Zika virus NAAT testing on a serum specimen, and Zika virus NAAT on a urine specimen, and
        - IgM testing for dengue only.

- Zika virus IgM testing is NOT recommended for symptomatic pregnant women.
  - Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.
  - There is notable cross-reactivity between dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive.
- If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results. If the dengue NAAT is positive, this provides adequate evidence of a dengue infection and no further testing is indicated.
- If the IgM antibody test for dengue is positive, this is adequate evidence of a dengue infection and no further testing is indicated.

- For symptomatic pregnant women who have had sex with someone who lives in or recently traveled to areas with a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset.
  - Only Zika NAAT should be performed.
  - If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results (CDC, 2019a)."

For pregnant women who have had a prenatal fetal ultrasound consistent with a Zika viral infection or who have traveled to an area during her pregnancy with a risk of Zika infections, the following recommendations are given by the CDC:

- "Zika virus NAAT and IgM testing should be performed on maternal serum and NAAT on maternal urine.
- If the Zika virus NAATs are negative and the IgM is positive, confirmatory PRNTs should be performed against Zika and dengue.
- If amniocentesis is being performed as part of clinical care, Zika virus NAAT testing of amniocentesis specimens should also be performed and results interpreted within the context of the limitations of amniotic fluid testing. It is unknown how sensitive or specific RNA NAAT testing of amniotic fluid is for congenital Zika virus infection or what proportion of infants born after infection will have abnormalities.
- Testing of placental and fetal tissues may also be considered (CDC, 2019a)."

Updated Recommendations for Diagnosis, Clinical Evaluation, and Management of Infants with Clinical Findings Consistent with Congenital Zika Syndrome Born to Mothers with Possible Zika Virus Exposure in Pregnancy

"Zika virus testing is recommended for infants with clinical findings consistent with congenital Zika syndrome and possible maternal Zika virus exposure during pregnancy, regardless of maternal testing results. Testing CSF for Zika virus RNA and Zika virus IgM antibodies should be considered, especially if serum and urine testing are negative and another etiology has not been identified (CDC, 2017c). *Updated*

Recommendations for Diagnosis, Clinical Evaluation, and Management of Infants without Clinical Findings Consistent with Congenital Zika Syndrome Born to Mothers with Laboratory Evidence of Possible Zika Virus Infection During Pregnancy

"Zika virus testing is recommended for infants without clinical findings consistent with congenital Zika syndrome born to mothers with laboratory evidence of possible Zika virus infection during pregnancy (CDC, 2017c)."

Updated Recommendations for Diagnosis, Clinical Evaluation, and Management of Infants without Clinical Findings Consistent with Congenital Zika Syndrome Born to Mothers with Possible Zika Virus Exposure in Pregnancy but without Laboratory Evidence of Possible Zika Virus Infection During Pregnancy

"This heterogeneous group includes mothers who were never tested during pregnancy as well as those whose test result could have been negative because of issues related to timing or sensitivity and specificity of the test. Because the latter issues are not easily discerned, all mothers with possible exposure to Zika virus during pregnancy who do not have laboratory evidence of possible Zika virus infection, including those who tested negative with currently available technology, should be considered in this group."

"Laboratory testing for congenital Zika virus infection is not routinely recommended for infants born to mothers in this category based on the unknown risk for infection; the lower likelihood of congenital Zika virus infection as a result of the declining prevalence of Zika virus infection; and limitations of infant laboratory testing. If abnormal findings are identified, these infants should receive further evaluation, including evaluation and testing for congenital Zika virus infection (CDC, 2017c)."

CDC Guidelines for Diagnostic Tests for Zika Virus (CDC, 2019b):

- *Molecular Test for Zika Virus – RNA NAT (nucleic acid testing):* This test is for symptomatic individuals within the first two weeks after symptom onset and for asymptomatic pregnant women who have traveled to areas with active Zika virus transmission. RNA NAT testing is also indicated for pregnant women who present for care ≥ 2 weeks after exposure and have been found to be IgM positive. A positive RNA NAT result confirms Zika virus infection and no additional testing is indicated, but a negative RNA NAT result does not exclude Zika virus infection and should be followed up with IgM antibody (serological) testing (CDC, 2019b).
- *Trioplex Real-time RT-PCR Assay:* The Trioplex rRT-PCR is a laboratory test designed to detect Zika virus, dengue virus, and chikungunya virus RNA. The Food and Drug Administration (FDA) has not cleared or approved this test. However, FDA has authorized the use of this test under an Emergency Use Authorization (EUA) (CDC, 2017b, 2019b).
- *Serologic Test for Zika Virus:* Zika virus-specific IgM and neutralizing antibodies typically develop toward the end of the first week of illness. IgM levels are variable, but generally are positive starting near day four post onset of symptoms and continuing for 12 weeks. Therefore, if RNA NAT is negative on serum and urine, serum IgM antibody testing for Zika, dengue, and chikungunya virus infections should be performed. In addition, serum samples collected ≥ 14 days after symptom onset, with no earlier samples collected, should be tested for anti-Zika virus, anti-dengue virus, and anti-chikungunya virus IgM antibodies (CDC, 2019b).

- *Zika MAC-ELISA:* Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) is used for the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid; however, due to cross-reaction with other flaviviruses and possible nonspecific reactivity, results may be difficult to interpret. This test cannot determine when an infection occurred, furthermore, positive, equivocal, or inconclusive tests must be confirmed by PRNT (CDC, 2019b).
- *Plaque Reduction Neutralization Test (PRNT):* Samples with presumptive positive, equivocal or inconclusive IgM antibody test result should be confirmed by PRNT, which measures virus-specific neutralizing antibodies to Zika virus and other endemic flaviviruses. PRNT must be conducted by CDC or a laboratory qualified by CDC (CDC, 2019b).

American Academy of Pediatrics (AAP) Report of the Committee of Infectious Diseases "Red Book"

The "Red Book" uses the CDC guidelines above (AAP, 2018).

The American College of Obstetricians and Gynecologists (ACOG) and Society of Maternal Fetal Medicine (SMFM)

In April 2017, ACOG and SMFM updated the practice advisory on Zika virus. It recommends that Zika virus testing should be done in the following situations:

- Non-pregnant women and all men with Zika virus exposure and symptoms consistent with Zika virus.
- Non-pregnant women and all men with Zika virus exposure but without symptoms consistent with Zika virus exposure.
- Pregnant women with Zika virus exposure should be tested regardless of symptom status.

ACOG and SMFM state that all pregnant women should be assessed for possible Zika virus exposure at each prenatal care visit. The practice advisory noted that "routine Zika virus testing is not currently recommended for women or men with possible Zika virus exposure without clinical illness who are attempting pregnancy". It further stated that "testing of specimens to assess risk for sexual transmission is currently not recommended" (ACOG, 2017).

ACOG also published a committee opinion regarding management of patients in the context of the Zika virus. In it, they list recommendations regarding testing, which are as follows:

- "Symptomatic pregnant women with possible Zika virus exposure or women who are pregnant with a fetus showing abnormalities consistent with congenital Zika virus syndrome should be tested as soon as possible. Asymptomatic pregnant women with ongoing possible exposure can be offered nucleic acid testing during pregnancy as part of routine obstetric care."
- "Asymptomatic pregnant women with possible Zika virus exposure but without ongoing possible exposure are not recommended routinely to have Zika virus testing, but testing can be considered as part of a shared patient–provider decision-making model."

This committee opinion was endorsed by the SMFM (ACOG, 2019).

World Health Organization (WHO)

The WHO recommends the following diagnostic strategies:

- NAT in patients presenting with onset of symptoms < 7 days
- Serology and/or NAT in patients presenting with onset of symptoms ≥ 7 days. Serology is the preferred method in specimens from patients with onset of symptoms >7 days (WHO, 2016b).
- The WHO also recommends the CDC's tests due to their superior sensitivity (WHO, 2016a).

The WHO also recommends this diagnostic strategy for pregnant women (WHO, 2016c).

International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)

The ISUOG has published guidelines regarding Zika virus during pregnancy. While it is noted that the interpretation of any laboratory testing methodologies is out of scope for these guidelines, the authors state that "National guidelines should be followed regarding testing. Expert opinion should be sought from national reference laboratories. In general, testing for ZIKV is possible in maternal serum by reverse transcription PCR (RT-PCR) or detection of ZIKV-specific IgM antibodies. The limitation of RT-PCR testing is that it can detect ZIKV only during, or immediately following, acute infection. ZIKV IgM testing is problematic because of cross-reactivity with other Flaviviruses and some immunizations. This may lead to an unreliably high false-positive rate of ZIKV serological testing, but negative serology results may be of value in 'ruling out' past ZIKV infection (Papageorghiou et al., 2016)."

Committee to Advise on Tropical Medicine and Travel (CATMAT)

The CATMAT published recommendations on Zika virus prevention and treatment. Regarding the routine testing of pregnant women, the CATMAT has stated that "Given the low risk of ZIKV infection, CATMAT recommends against routine testing of asymptomatic pregnant women. The poor positive predictive value, especially for screening serology tests, means a positive test has a high likelihood of being a false positive, which may have significant adverse consequences. The low population prevalence of infection means a negative test result is of negligible clinical utility (CATMAT, 2019, 2020)."

In a symptomatic traveler, "Diagnostic testing can be considered after discussion of the risks of both false negative and false positive results (CATMAT, 2019, 2020)." For asymptomatic travelers, routine testing is not recommended.

Regarding screening and management, CATMAT recommends the following:

- "Testing for ZIKV infection using PCR should be considered in the diagnosis of any ill traveller with compatible epidemiologic and clinical history, when symptom onset is within 3 days after arrival in, to 14 days after departing from an area of risk as identified by the WHO.
- "Given the low incidence of infection in most regions, most testing should be limited to molecular techniques, performed within approximately 10 days of the onset of symptoms. It may often be appropriate to perform molecular tests for other similar arboviral infections on the same specimen."
- "Serologic testing could be considered in exceptional circumstances for male returned travellers from areas of risk (as designated by the WHO) whose clinically compatible illness has resolved, and

are at least 2 weeks post exposure, in order to help assess for potential contagiousness to sexual partners when it is impossible or dangerous to delay attempts at conception."

- "Serological testing of male individuals with a history of travel to an area of risk (as designated by the WHO) but no history of related symptoms is not recommended, given the extremely low risk of infection and high risk of false positive serology."
- "Testing should be offered to pregnant women with acute signs and symptoms compatible with ZIKV. Given the reports of longer periods of viremia in some pregnant women, for the patient with symptoms during the preceding 12 weeks, RT-PCR (on blood and urine) is the preferred testing modality. Serology is not recommended for routine testing and should only be requested very judiciously as it is not appropriate in most cases. For the convalescent patient with symptom onset over 12 weeks ago, RT-PCR will be of minimal value…a woman whose fetus is suspected of having a congenital anomaly should also be offered testing if she or her partner has travelled to any location where ZIKV transmission may be occurring even at a low level."
- "Infants born to women with confirmed or suspected ZIKV infection in pregnancy, or those with unexplained microcephaly, intracranial calcifications, ventriculomegaly or major structural central nervous system abnormalities or other symptoms of congenital ZIKV infection in whom the mother had potential exposure to the virus, should be tested. This testing should include serology, PCR of serum (umbilical cord or infant sample), and PCR of placenta; if CSF is sampled, this can also be sent for PCR and serology (CATMAT, 2019, 2020)."

Canadian Paediatric Society (CPS)

The CPS guidelines on Zika virus state that a diagnosis can be made by an IgM or IgG neutralizing antibody, or by PCR through the detection of Zika virus RNA. These testing methods may be utilized if an individual fits into the following categories:

- "Child born from 2016 on with unexplained microcephaly (present at birth or detected later), intracranial calcifications, ventriculomegaly or major structural CNS abnormalities AND maternal history of:
  - Travel to a ZIKA-endemic country during pregnancy, or
  - Sexual contact during pregnancy with a male who travelled to a ZIKA-endemic country in the preceding 6 months (Robinson, 2017)."

Finally, "Testing is generally not advised for asymptomatic or symptomatic children with exposure to ZIKV [Zika virus] after birth, unless they require hospitalization (Robinson, 2017)."

Public Health England (PHE)

The PHE published guidance on who to test for Zika virus infection and which samples to collect.

The PHE states that the test is not available "for individuals who have had no symptoms suggestive of Zika infection", including:

- "asymptomatic pregnant women who have travelled from Zika-affected countries"
- "asymptomatic returned male travelers whose partners are currently pregnant"

- "asymptomatic returned male and female travelers who are trying to conceive".

The PHE writes that Zika virus infection should be considered in the following circumstances:

- "any patient who has, or has had, a rash illness or other symptoms suggestive of Zika virus infection, that began whilst in any country or area with risk of Zika virus transmission, or within 2 weeks of leaving that country".
- "any patient presenting with typical Zika-like symptoms apparently due to sexual transmission; that is, there is no history of travel a Zika-affected country or the symptoms began more than 2 weeks after travel to a Zika-affected country, and their male sexual partner had travelled within the last 3 months from a country or area with risk of Zika virus transmission".

Overall, the PHE testing algorithm does not contain a route for testing without presence of symptoms suggestive of the Zika virus infection (PHE, 2019).

Royal College of Obstetricians and Gynaecologists (RCOG)/Royal College of Midwives (RCM)/PHE/Health Protection Scotland (HPS)

This joint guideline outlines statements for Zika virus in pregnancy.

- "The diagnosis of Zika virus infection should be considered in individuals who experience symptoms suggestive of acute Zika virus infection within 2 weeks of leaving an area with risk for Zika virus transmission OR within 2 weeks of sexual contact with a male sexual partner who has recently travelled within the previous three months to an area with high or moderate risk of Zika virus transmission."
- "Pregnant women presenting to their healthcare provider with current or previous symptoms of Zika virus that began within 2 weeks of return to the UK, should be tested."
- "Zika virus testing is not available for individuals who do not have symptoms consistent with Zika virus infection" (RCOG/RCM/PHE/HPS, 2019).

American Society for Microbiology (ASM)

The ASM released guidelines in 2016 on laboratory testing for Zika virus. They state, "Diagnostic testing may be warranted for patients who live in or have recently travelled to an endemic region and are critically ill, hospitalized or pregnant, or infants born to Zika virus positive mothers." The ASM endorses CDC guidelines on Zika as well.

In terms of recommended laboratory testing, the ASM stated the following:

- "For pregnant women ***with*** a clinical illness consistent with Zika within 2 weeks of travel to areas with documented Zika virus transmission:
    - Zika virus reverse transcription polymerase chain reaction (RT-PCR) on maternal serum. This is recommended during the first 7 days following symptom onset; however, the sensitivity of RT-PCR decreases significantly after 5-7 days. Urine PCR testing may extend the period during which RNA is reliably detected up to 2 weeks after symptom onset.

- IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA IgM) testing with confirmation by a neutralizing antibody assay on maternal serum. Sera should be collected ≥4 days after symptom onset.

- For pregnant women ***without*** symptoms, but with travel history within 2 weeks to areas with documented Zika virus transmission, or sexual contact with an individual with an appropriate travel history and clinical illness consistent with Zika infection:
  - MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on maternal serum. Sera should be collected ≥4 days after symptom onset.
- For cases where fetal microcephaly or intracranial calcifications are observed on prenatal ultrasound:
  - MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on maternal serum.
  - An amniocentesis may be considered, depending on gestational age. Zika virus RT-PCR can be performed on amniotic fluid.
- For infants with microcephaly or intracranial calcifications or infants whose mothers have positive or inconclusive test results for Zika virus (potential congenital infection):
  - Zika virus RT-PCR on infant serum.
  - MAC-ELISA Ig M testing with confirmation by a neutralizing antibody assay on infant serum.
    - The initial sample should be collected either from the umbilical cord or directly from the infant within 2 days of birth, if possible.
  - If cerebrospinal fluid is obtained for other studies, test for Zika virus RNA, Zika virus IgM and neutralizing antibodies.
  - If not already performed during pregnancy, test mother's serum for Zika virus IgM and neutralizing antibodies.
  - Consider histopathologic evaluation of the placenta and umbilical cord with Zika virus immunohistochemical staining on fixed tissue and Zika virus RT-PCR on fixed and frozen tissue."

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On October 5, 2017 the FDA approved the cobas Zika for use to screen donor samples for Zika virus RNA in plasma samples from individual human donors, including donors of whole blood and blood components, and other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating. The clinical sensitivity and specificity was evaluated at 100% (25 samples) and 99.997% (358024 samples) respectively (FDA, 2017).

On July 5, 2018, the FDA approved the Procleix Zika Virus Assay by Grifols. The test description is as follows: "The Procleix Zika Virus Assay is a qualitative in vitro nucleic acid test for the detection of Zika virus (ZIKV) RNA in plasma specimens from individual human donors, including volunteer donors of whole blood and blood components, for transfusion. It is also intended for use in testing plasma or serum specimens to screen other living (heart-beating) donors of organs and Human Cells, Tissues, and

Cellular and Tissue-Based Products (HCT/Ps), and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens. The assay is intended for use in testing individual donor samples. It is also intended for use in testing pools of human plasma composed of equal aliquots of not more than 16 individual specimens from volunteer donors of whole blood components. This assay is not intended for use as an aid in diagnosis of Zika virus infection." The specificity was evaluated at 100%, and the sensitivity was evaluated as low as 10 IU/mL (10 IU/mL was the lowest concentration the assay had a 100% detection rate on) (FDA, 2018). Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86794	Zika virus, IgM
87662	Infectious agent detection by nucleic acid (DNA or RNA); Zika virus, amplified probe technique

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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ZIKA Virus Risk Assessment, continued

IX. Revision History

Revision Date	Summary of Changes
4/27/22	The following modifications were made: Update wording throughout coverage criteria to align with updated clinical standards; removed the term RNA, and replaced the term NAT with NAAT throughout coverage criteria; added coverage criteria #2: Zika virus NAAT testing of maternal serum and urine specimens MEETS COVERAGE CRITERIA in the following situations: a. pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus b. symptomatic pregnant individuals who have had exposure to Zika virus during pregnancy c. asymptomatic pregnant individuals who have had exposure to Zika virus via travel to an area of risk outside of U.S. territories for a time limit of up to 12 weeks post-travel.; added coverage criteria #3: In pregnant individuals with possible exposure to Zika virus AND who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection, Zika virus IgM testing of maternal serum MEETS COVERAGE CRITERIA; and removed the following coverage criteria: "Zika virus serum IgM testing DOES NOT MEET COVERAGE CRITERIA in symptomatic pregnant women."