Select Health

Select Health Medical Policies

Laboratory Utilization Policies—Part 1

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β-Hemolytic Streptococcus Testing

Policy #: AHS – G2159	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 3/22/23 (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

Streptococcus are Gram-positive, catalase-negative bacteria that are further divided into α-hemolytic, such as *S. pneumoniae* and *S. mutans*; β-hemolytic, such as *S. pyogenes* (Group A), *S. agalactiae* (Group B), and *S. dysgalactiae subsp equisimilis* (Groups C and G); and γ-hemolytic, such as *Enterococcus faecalis* and *E. faecium* (Wessels, 2022). Streptococcal infections can be manifested in a variety of pathologies, including cutaneous infections, pharyngitis, acute rheumatic fever, pneumonia, postpartum endometritis, and toxic shock syndrome to name a few. Streptococcal infections can be identified using bacterial cultures obtained from blood, saliva, pus, mucosal, and skin samples as well as rapid antigen diagnostic testing (RADT) and nucleic acid-based methodologies (Chow, 2022; Wessels, 2022).

Note: For prenatal screening of Group B Streptococcus, please review policy AHS-G2035.

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)

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.

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1. For the detection of a streptococcal infection causing respiratory illness, bacterial culture testing from a throat swab **MEETS COVERAGE CRITERIA** when one of the following conditions is met:
    a. When the individual has a modified Centor criteria score of 3 or greater (See Note 1 below).
    b. When the individual is suspected of having bacterial pharyngitis in the absence of viral features, including cough, oral ulcers, and rhinorrhea.
    c. Following a negative rapid antigen diagnostic test (RADT) in a symptomatic child or adolescent.
2. Blood culture testing for a streptococcal infection **MEETS COVERAGE CRITERIA** when one of the following conditions is met:
    a. In individuals who fail to demonstrate clinical improvement and in those who have progressive symptoms or clinical deterioration after initiation of antibiotic therapy.
    b. In cases of suspected prosthetic joint infection.
3. In cases of skin and/or soft tissue infections, bacterial culture testing for a streptococcal infection from a skin swab or from pus **MEETS COVERAGE CRITERIA.**
4. In cases of suspected viral pharyngitis, bacterial culture testing for streptococci from a throat swab **DOES NOT MEET COVERAGE CRITERIA**.
5. Except in cases of asymptomatic children under the age of three years who have a mitigating circumstance (including a symptomatic family member), RADT for a streptococcal infection **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:
    a. As a follow-up test to either a positive or negative bacterial culture test for a streptococcal infection.
    b. As a screening method in an asymptomatic patient.
    c. In cases of suspected viral pharyngitis.
6. Except in cases of suspected acute rheumatic fever (ARF) or post-streptococcal glomerulonephritis (PGSN), serological titer testing **MEETS COVERAGE CRITERIA**.
7. The simultaneous coding for BOTH amplification and direct probes **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.

8. The following tests **DO NOT MEET COVERAGE CRITERIA:**
    a. Panel tests that screen and identify multiple streptococcal strains (*S. pyogenes* [group A], *S. agalactiae* [group B], *S. dysgalactiae* [groups C/G], α-hemolytic streptococcus, and/or γ-

hemolytic streptococcus), using either immunoassay or nucleic acid-based assays, such as the Solana Strep Complete Assay and the Lyra Direct Strep Assay.

b. MALDI-TOF identification of streptococcus.

c. Anti-streptolysin O immunoassay (EXCEPT in cases of suspected ARF or PSGN).

d. The quantification of any strain of streptococcus using nucleic acid amplification, including PCR.

e. Hyaluronidase activity or anti-hyaluronidase immunoassay (EXCEPT in cases of suspected ARF or PSGN); OR

f. Streptokinase activity or anti-streptokinase immunoassay (EXCEPT in cases of suspected ARF or PSGN).

g. Nicotinamide-adenine dinucleotidase activity or anti-nicotinamide-adenine immunoassay.

Note 1: Centor criteria includes tonsillar exudates, tender anterior cervical lymphadenopathy, fever, and absence of cough with each criterion being worth one point (Chow, 2022).

IV. Scientific Background

Bacterial acute pharyngitis is caused most often by a Group A Streptococcus (*S. pyogenes* or GAS), accounting for 5-15% of all acute pharyngitis cases in adults. Group C or Group G Streptococcus (*S. dysgalactiae subsp equisimilis* or GCS/GGS) is believed to be a causative agent in 5-10% of the cases of pharyngitis; however, "pharyngitis cause group C or G Streptococcus is clinically indistinguishable from GAS pharyngitis" but is more common in young adults and college students (Chow, 2022). "Diagnosis of infection due to group C streptococci (GCS) and group G streptococci (GGS) depends on identification of the organism in a culture from a clinical specimen. In general, a positive culture from a normally sterile site, such as blood, synovial fluid, or cerebrospinal fluid (CSF), can be considered definitive evidence of infection in the setting of a compatible clinical syndrome. The interpretation of positive cultures for GCS or GGS from the pharynx or from cutaneous sites such as open ulcers or wounds is less straightforward since asymptomatic colonization of the upper airway and skin also occurs (Wessels, 2022)." GAS occurs most frequently in the very young and the elderly; although, GAS infections can occur in any age-group. The rates of severe GAS infections have been increasing in the United States as well as in other developed nations (Schwartz et al., 1990).

The Centor criteria can be used to gauge the likelihood of pharyngitis due to a GAS infection. The four components of the Centor criteria are tonsillar exudates, tender anterior cervical lymphadenopathy, fever, and absence of cough with each criterion being worth one point. Patients who score less than three according to the Centor criteria are unlikely to have pharyngitis due to GAS and do not require strep testing or antibiotics; patients scoring ≥3 can be tested for GAS pharyngitis (Chow, 2022).

GAS is associated with bacterial pharyngitis, scarlet fever, acute rheumatic fever, and post-streptococcal glomerulonephritis. Group A strep pharyngitis presents as a sudden-onset of sore throat with

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odynophagia and fever; it is commonly referred to as “strep throat”. In children, additional symptoms can include abdominal pain, nausea, and vomiting. Viral pharyngitis, which accounts for more than 80% of pharyngitis, typically presents with cough, rhinorrhea, hoarseness, oral ulcers, and conjunctivitis unlike GAS pharyngitis. Rare cases of mucopurulent rhinitis caused by GAS has been reported in children under the age of three (CDC, 2022b). Scarlet fever can accompany strep throat. Besides the typical erythematous rash that typically begins on the trunk before spreading outward, scarlet fever can also present as a flushed face, “and the area around the mouth may appear pale (i.e., circumoral pallor).” “Strawberry tongue” can occur due to “yellowish white coating with red papillae” (CDC, 2022d). Scarlet fever is more easily transmitted than asymptomatic carriers through saliva and nasal secretions. Acute Rheumatic Fever (AFR), besides the characteristic fever, can affect the cardiovascular system (carditis and valvulitis), the musculoskeletal system (arthritis), the integumentary system (subcutaneous nodules and erythema marginatum), and the central nervous system (chorea). “Inadequate or lack of antibiotic treatment of streptococcal pharyngitis increases the risk of someone developing acute rheumatic fever. In approximately one-third of patients, acute rheumatic fever follows subclinical streptococcal infections or infections for which medical attention was not sought (CDC, 2022a).” Post-streptococcal glomerulonephritis (PSGN) presents with edema, hypertension, proteinuria, macroscopic hematuria, lethargy, and, at times, anorexia. “Laboratory examination usually reveals mild normocytic normochromic anemia, slight hypoproteinemia, elevated blood urea nitrogen and creatinine, elevated erythrocyte sedimentation rate, and low total hemolytic complement and C3 complement.” Urine output is usually decreased, and urine examination “often reveals protein (usually <3 grams per day) and hemoglobin with red blood cell casts (CDC, 2022c).”

The virulence factors of GAS include M proteins, a group of more than 80 known proteins that protein the bacteria against phagocytosis; streptolysin O, a thiol-activated cytolysin; hyaluronidase, which hydrolyzes hyaluronic acid within the host tissue; streptokinase, an enzyme that activates plasmin; nicotinamide-adenine dinucleotidase (NADase), a glycohydrolase of uncertain function; and deoxyribonucleases (DNases) A, B, C, and D. Streptolysin O bind to the eukaryotic membrane’s cholesterol to facilitate the characteristic cellular lysis of a GAS infection. Cholesterol and anti-streptolysis O (ASO) antibodies can mitigate streptolysin O damage, and ASO titers often increase following an infection with the peak occurring around four to five weeks post-infection. “Nonsuppurative complications such as rheumatic fever and poststreptococcal glomerulonephritis generally develop during the second or third week of illness… About 80 percent of patients with acute rheumatic fever or poststreptococcal glomerulonephritis demonstrate a rise in ASO titer; however, the degree of ASO titer elevation does not correlate with severity of disease. In patients with suspected rheumatic fever or glomerulonephritis but with an undetectable ASO titer, prompt testing for other antistreptococcal antibodies such as anti-DNase B (detectable for six to nine months following infection), streptokinase, and antihyaluronidase should be performed.” (Stevens & Bryant, 2022)

Acute rheumatic fever (ARF) can occur two to four weeks following GAS pharyngitis. The five major manifestations of ARF are carditis and valvulitis (up to 70% of patients exhibit this condition with ARF), arthritis (up to 66%), CNS system involvement (10-30%), subcutaneous nodules (0-10%), and erythema marginatum (<6%) (Steer & Gibofsky, 2022). A diagnosis of ARF is not predicated by confirmation of a

preceding GAS infection; however, it is helpful, especially in diagnosing children and young adults with arthritis and/or carditis. Evidence of GAS should include either a positive throat culture, a positive RADT, or an elevated or rising titer of either ASO or anti-DNase B. These two antibodies are used frequently in clinical practice due to their high sensitivity in diagnosing streptococcal infections (Steer & Gibofsky, 2022; Steer et al., 2015). A study by Blyth and Robertson demonstrated that the sensitivity of using only a single antibody in the diagnosis of streptococcus ranged from 70.5-72.7%; however, the combination of ASO and anti-DNase B increased the specificity to 88.6% with a sensitivity of 95.5%. The addition of anti-streptokinase (ASK) did not increase either the sensitivity or specificity of testing (Blyth & Robertson, 2006).

A study in Norway in 2013 show that necrotizing soft tissue infections can be caused by GAS or GGS/GCS. The mean annual incidence rate is 1.4 per 100,000. During the time period studied (2000-2009), 61 cases of necrotizing soft tissue infections in Norway were due to GAS while nine cases were due to GCS/GGS. "Our findings indicate a high frequency of streptococcal necrotizing fasciitis in our community. GCS/GGS infections contribute to the disease burden but differ from GAS cases in frequency and predisposing factors." They note that "the GCS/GGS patients were older, had comorbidities more often and had anatomically more superficial disease than the GAS patients (Bruun et al., 2013)." A review in 2014 also noted the population most affected by GCS/GGS, but they note that "the case fatality in bacteremia has been reported to be 15-18% (Rantala, 2014)."

Group B Streptococcus (GBS) is frequently found in human gastrointestinal tracts and genitalia and can be spread to the upper respiratory tract of newborns. In neonates, a GBS infections can cause bacteremia, pneumonia, meningitis, and sepsis. GBS can also cause complications in pregnancy, such as urinary tract infections and chorioamnionitis. GBS, in pregnant and postpartum women, is of special concern since it is implicated in up to 31% of cases of bacteremia without a focus, 8% of postpartum endometritis, and 2% of pneumonia; moreover, if left unchecked, GBS can also result in preterm labor and miscarriage. In the adult population at large, GBS infections can be manifest as soft tissue infections, sepsis, and bacteremia (Barshak, 2021; Puopolo, et al., 2021). "Invasive disease in infants is categorized on the basis of chronologic age at onset. Early-onset disease usually occurs within the first 24 hours of life (range, 0 through 6 days) and is characterized by signs of systemic infection, respiratory distress, apnea, shock, pneumonia, and less often, meningitis (5%–10% of cases). Late-onset disease, which typically occurs at 3 to 4 weeks of age (range, 7 through 89 days), commonly manifests as occult bacteremia or meningitis (approximately 30% of cases); other focal infections, such as osteomyelitis, septic arthritis, necrotizing fasciitis, pneumonia, adenitis, and cellulitis, occur less commonly. Nearly 50% of survivors of early- or late-onset meningitis have long-term neurologic sequelae (encephalomalacia, cortical blindness, cerebral palsy, visual impairment, hearing deficits, or learning disabilities). Late, late-onset disease occurs at 90 days of age and beyond, usually in very preterm infants requiring prolonged hospitalization (Pediatrics, 2018)."

Type of Testing

β-Hemolytic Streptococcus Testing, continued

Test	Description	Rationale
Culture	Cultures can be taken from a swab of the affected tissue when possible, such as the back of the throat and tonsils (1). The cultures are typically grown on a solid, complex rich medium such as Trypticase Soy Agar (TSA) supplemented with 5% sheep blood so that the zone of b-hemolysis can easily be visualized (2). Culture testing can be supplemented with additional conventional identification tests, such as the Lancefield antigen determination test and the PYR test (3).	The CDC considers the throat culture the 'gold standard' (4). This testing method can be time intensive. "Throat culture also can identify other bacteria that cause pharyngitis less commonly than GAS (eg, group C and group G streptococci, *Arcanobacterium haemolyticum*). However, most laboratories do not routinely identify these pathogens in throat cultures unless specifically requested to do so (5)."
Serology	Many possible serological tests can be performed, including a measurement of the antibody titers associated with a streptococcal infection. Virulence factors that can be monitored include hyaluronidase, streptokinase, nicotinamide-adenine dinucleotidase, DNase B, and streptolysin O. DNase B and streptolysin O are more frequently used in clinical practice (6).	Anti-streptococcal antibody titers represent past infections and should not be used to routinely diagnose an acute infection (7). Antistreptolysin O (ASO) and/or anti-DNase B (ADB) testing can be used to determine prior streptococcal infection associated with disorders such as rheumatic fever and glomerulonephritis. "An increase in titer from acute to convalescent (at least two weeks apart) is considered the best evidence of antecedent GAS infection. The antibody response of ASO peaks at approximately three to five weeks following GAS pharyngitis, which usually is during the first to third week of ARF, while ADB titers peak at six to eight weeks (8)." Antibody titers are dependent on the age of the patients with children having considerably higher 'normal' levels than adults due to frequent exposure to *S. pyrogenes* (3).
Rapid Antigen Diagnostic Testing (RADT)	RADTs can be performed on a swab at the point of care or can be transported to a lab for testing (9). Numerous RADTs directly detect antigens through an agglutination method or the use of immunoassays, including enzyme-based assays, optical assays, and liposome-based assays that are commercially available (3).	Many RADTs are commercially available but can vary considerably in specificity, sensitivity, and ease of use. "In pediatric patients, if the direct antigen test is negative, and if the direct antigen test is known to have a sensitivity of <80%, a second throat swab should be examined by a more sensitive direct NAAT or by culture as a means of arbitrating possible false-negative direct antigen test results. This secondary testing is not necessarily required in adults. A convenient means of facilitating this 2-step algorithm of testing for Streptococcus pyogenes in pediatric patients is to collect a dual swab initially, recognizing that the second swab will be discarded if the direct antigen test is positive (9)."
Nucleic Acid Amplification	NAATs amplify DNA or RNA to detect the presence of microorganisms. Some are	More sensitive than antibody-based testing for streptococcus. Direct NAATs usually require the use

β-Hemolytic Streptococcus Testing, continued

Test	Description	Rationale
Tests (NAATs)	offered as point-of-care (POC) rapid diagnostic tests while others require special laboratory equipment (9). Some NAATs utilize real-time polymerase chain reaction (rt-PCR), such as the Lyra Direct Strep Assay, while others use a helicase-dependent amplification (HDA)-based methodology like the Solana Strep Complete assay. NAATs are often qualitative but specific NAATs can be quantitative. NAATs can vary in their selectivity, sensitivity, and ability to differentiate between strains of streptococci.	of enriched broth cultures. "Negative direct NAAT results do not have to be arbitrated by a secondary test (9)."
Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)	MALDI-TOF mass spectrometry can be used to quickly identify both gram-negative and gram-positive bacteria once the organism is available in a pure culture on solid medium. The results of the MALDI-TOF test is compared to a known database of spectra of microorganisms for identification (10).	"For less common organisms, the MALDI-TOF result may not be conclusive, and additional bench tests or molecular tests may be required (10)."

(1) Reference: (AACC, 2015)
(2) Reference: (Gera & McIver, 2013)
(3) Reference: (Spellerberg & Brandt, 2016)
(4) Reference: (CDC, 2022b)
(5) Reference: (Wald, 2022)
(6) Reference: (Stevens & Bryant, 2022)
(7) Reference: (Shulman et al., 2012)
(8) Reference: (A. Steer & Gibofsky, 2022)
(9) Reference: (Miller et al., 2018b)
(10) Reference: (Freeman & Roberts, 2021)

Clinical Utility and Validity

Rapid in vitro diagnostic tests (RIDT), such as the Alere I Strep A, have been CLIA-waived by the FDA. These tests provide results more quickly than the traditional "gold standard" bacterial culture testing. A 2018 study comparing rapid antigen GAS testing, the Alere I Strep A test—an RIDT using isothermal nucleic acid amplification, and throat cultures. "The sensitivity and specificity of the molecular test were 98% and 100%, respectively, compared with culture. There was a 9% false-positive rate with the rapid antigen-based testing…. The Alere test is sufficiently sensitive and specific for definitive GAS testing in a pediatric urgent care setting (Weinzierl et al., 2018)." In 2016, Cohen et al extensively reviewed the use of rapid antigen detection tests (RADT) for GAS in children. They reviewed 98 unique studies consisting of a total of 101,121 participants and compared both major types of RADTs—enzyme immunoassays (EIA) and optical immunoassays (OIA). "RADT had a summary sensitivity of 85.6%...There was substantial heterogeneity in sensitivity across studies; specificity was more stable. There was no trade-off between sensitivity and specificity….The sensitivity of EIA and OIA tests was comparable (summary sensitivity 85.4% versus 86.2%)… Based on these results, we would expect that amongst 100 children with strep throat, 86 would be correctly detected with the rapid test while 14 would be missed and not receive antibiotic treatment (Cohen et al., 2016)." Another multicenter study using the Alere I Strep A test on

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cultures obtained from 481 patients of all ages show that the RIDT had 96.0% sensitivity and 94.6% specificity. The authors conclude that this "could provide a one-step, rapid, point-of-care testing method for GAS pharyngitis and obviate backup testing on negative results (Cohen et al., 2015)." This study did note that there are newer tests available that have higher sensitivity, but these tests require more time than the Alere I Strep A method.

Due to the time constraints of clinical laboratories and the variability of RADTs, nucleic acid amplification test (NAAT) use has been increasing in clinical settings. The FDA has approved multiple NAATs for the detection of Streptococcus. The Lyra Direct strep assay is an FDA-approved, NAAT that uses real-time PCR to qualitatively detect the presence of GAS and GGS/GCS in throat swab samples. It should be noted, though, that this assay does not distinguish between GGS and GCS. A study by Boyanton *et al*. evaluated the efficacy of the Lyra Direct method as compared to the traditional, time-consuming culture test for GAS and GGS/GCS. The sample sizes were not large (n = 19 for GAS and n = 5 for GGS/GCS out of a total of 161 samples submitted); however, the Lyra Direct strep assay did correctly detect "all b-hemolytic streptococci..." and "in batch mode, the Lyra assay reduced intra-laboratory turnaround time by 60% (18.1 h versus 45.0 h) but increased hands-on time by 96% (3 min 16 s versus 1 min 40 s per specimen) (Boyanton et al., 2016)." The authors note that the RADTs "have largely augmented bacterial culture (the gold standard). However, the performance of commercially available [RADTs] varies greatly depending upon the manufacturer, methodology used (i.e., optical immunoassay, immunochromatographic, or enzyme immunoassay), and the patient population (i.e., pediatric versus adult) being tested. Due to these limitations, nucleic acid amplification tests (NAATs) are being implemented in clinical laboratories (Boyanton et al., 2016)." The Solana method is also an FDA-approved NAAT, but it uses a rapid helicase-dependent amplification (HDA) methodology. Solana is available for either GAS testing or as a panel testing for GAS, GCS, and GGS. A study by Uphoff and colleagues compared the Solana GAS testing to that of conventional culture testing. Their research used 1082 throat swab specimens. The traditional culture tested positive in 20.7% of the samples as compared to 22.6% positive values in the HDA-based methodology. The Solana assay in their results had 98.2% sensitivity and 97.2% specificity. "In 35 min, the HDA method provided rapid, sensitive GAS detection, making culture confirmation unnecessary (Uphoff et al., 2016)." Recently, another study compared an HDA-based method to the Simplex GAS Direct PCR-based method, which is another FDA-approved diagnostic test. The Simplex GAS Direct method does not require initial DNA extraction from the sample, a potential time-saving benefit. The study used 289 throat swabs. The HDA- based method "compared to Simplexa qPCR had sensitivity, specificity, positive predictive value and negative predictive value of 93.1% vs 100%, 100% vs. 100%, 100% vs. 100% and 98.31% vs. 100% respectively… Simplexa qPCR has improved performance and diagnostic efficiency in a high-volume laboratory compared to [HDA-based method] for GAS detection in throat swabs (Church et al., 2018)."

The Solana® Strep Complete Assay by Quidel received FDA clearance in 2016. According to Quidel's FDA application, it is defined as "a rapid in vitro diagnostic test, using isothermal amplification technology (helicase-dependent amplification, HDA) for the qualitative detection and differentiation of Streptococcus pyogenes (Group A β-hemolytic Streptococcus) and Streptococcus dysgalactiae (pyogenic Group C and G β-hemolytic Streptococcus) nucleic acids isolated from throat swab specimens obtained

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from patients with signs and symptoms of pharyngitis, such as sore throat (Lollar, 2016)." This test must be performed using Quidel's Solana proprietary equipment. According to the 510(k) application, the Solana Strep Complete Assay panel has a clinical sensitivity and specificity for GAS of 98.8% and 98.9%, respectively, as compared to the Lyra Direct Strep Assay's reported 96.5% sensitivity and 98.0% specificity for GAS. The Lyra Direct Strep Assay is a real-time PCR-based assay that cannot differentiate between the pyogenic strains of streptococci. Concerning the pyrogenic GCS/GGS, the Solana Strep Complete Assay panel has a clinical sensitivity of 100% with a specificity of 99.5% as compared to Lyra Direct Strep Assay's reported 95.7% sensitivity and 98.3% specificity for GCS/GGS strains. The reported testing time also varies between the two assays with Solana requiring 25 minutes versus 60-70 minutes for the Lyra Direct Strep Assay (Lollar, 2016).

A recent study by Helmig and Gertsen evaluated the accuracy of PCR-based testing for GBS in pregnant women. Their study used rectovaginal swabs from 106 women in gestational weeks 35-37. For each, both a GBC culture and a PCR-based molecular GBS test (Xpert GBS of Cepheid Ltd) were performed. Only one PCR test yielded no result, so the invalid PCR-based test rate is <1%. 25/106 of the GBS cultures tested positive as compared to 27/105 of the PCR-based test. The specificity of the PCR-based test was 97.5% with a 100% sensitivity and a 92.6% positive predictive value. The authors conclude that "the PCR test has sufficient accuracy to direct intrapartum antibiotic prophylaxis for GBS transmission during delivery (Helmig & Gertsen, 2017)." A preliminary study in France of 1416 mothers with newborns compared swab cultures and GBS PCR assay for their predictive value of early-onset bacterial sepsis (EOS) in newborns since GBS is the most common cause of EOS. The results show that "the diagnostic values of the two tests highlighted a nonsignificant superiority of intrapartum GBS PCR assay" but that "the negative predictive value was improved with intrapartum PCR assay (negative likelihood ratio [LR]: 0.3 [0.1-0.9] vs. 0.6 [0.4-1.1])…. These results suggest that the intrapartum GBS PCR assay offers a better predictive value of GBS EOS that the usual vaginal culture swab at the 9th month but requires confirmation by large studies (Raignoux et al., 2016)."

Luo et al. "evaluated the overall diagnosis and treatment of acute pharyngitis in the United States, including predictors of test type and antibiotic prescription". Five categories of tests were identified, which were RADT [rapid antigen detection test], RADT plus culture, other tests, nucleic acid amplification testing (NAAT), and no test. Pharyngitis events from 2011-2015 were examined and a total of 18.8 million pharyngitis events across 11.6 million patients were included. 68.2% of events were found to occur once, with 29.1% requiring further follow-up. 43% of events were diagnosed by RADT and 20% were diagnosed by RADT plus culture. NAAT testing also increased 3.5-fold from 2011-2015 (going from 0.06% to 0.27%). Antibiotics were used in 49.3% of events as a whole. For RADT plus culture, antibiotics were used 31.2% of the time, for NAAT alone, 34.5%, for RADT alone, 54.2%, for no test, 57.1%. The authors concluded that "Diagnostic testing can help lower the incidence of inappropriate antibiotic use, and inclusion of NAAT in the clinical guidelines for GAS pharyngitis warrants consideration." (Luo et al., 2019)

Baptista de O Luiz et al. evaluated the "prevalence and persistence of beta-haemolytic streptococci throat carriage and type the bacterial population". A total of 121 children and 127 young adult volunteers contributed throat swabs (for culture), and these volunteers were screened quarterly for

β-Hemolytic Streptococcus Testing, continued

beta-haemolytic bacterial species. Carriage was detected in 34 volunteers (13.7%). Seventeen children were found to carry Group A *Streptococcus*, while seventeen young adults were found to carry four separate subspecies (*Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE), *Streptococcus pyogenes*, *Streptococcus agalactiae* and the *Streptococcus anginosus* group). The authors also identified persistent carriage for as long as 6 months in two children and for as long as 1 year in three young adults. The authors concluded that "prevalence was slightly greater among children, but persistent carriage was greater among young adults, with SDSE being the species most associated with persistence." (FB et al., 2019)

Fraser et al. performed a meta-analysis to assess the cost-effectiveness of point-of-care testing for detection of Group A Streptococcus. The authors remarked that this type of testing has seen increased use as an adjunct for managing care, such as for prescribing antibiotics. Thirty-eight studies of clinical effectiveness were included, along with three studies of cost-effectiveness. Twenty-six articles "reported on the test accuracy of point-of-care tests and/or clinical scores with biological culture as a reference standard". Overall, 21 point-of-care tests were evaluated. The authors identified two populations of interest; "patients with Centor/McIsaac scores of ≥ 3 points or FeverPAIN scores of ≥ 4 points". Test sensitivity for these populations ranged from 0.829-0.946 while test specificity ranged from 0.849-0.991. However, the authors did note there was significant heterogeneity and expressed doubts that any single study "accurately captured a test's true performance". The authors developed an economic model to explore the cost-effectiveness of this type of testing, and 14 of the 21 tests were included in this model. Per the current National Institute for Health and Care Excellence's cost-effectiveness thresholds, these tests were not found to be cost-effective. The authors acknowledged significant uncertainties in the estimates, such as penalties for antibiotic over-prescriptions. The authors concluded that "the systematic review and the cost-effectiveness models identified uncertainties around the adoption of point-of-care tests in primary and secondary care settings. Although sensitivity and specificity estimates are promising, we have little information to establish the most accurate point-of-care test." (Fraser et al., 2020; Kim et al., 2019)

Bilir et al. (2021) studied the cost-effectiveness of point of care nucleic acid amplification tests (NAAT) for streptococcus in the US. Point of care NAAT was compared to rapid antigen detection tests (RADT) and culture. Costs, clinical effects, antibiotic complications, number of patients treated, and antibiotic utilization were studied. Analysis showed that the POC NAAT method would cost $44 per patient while RADT and culture would cost $78 per patient. "Compared with RADT + culture, POC NAAT would increase the number of appropriately treated patients and avert unnecessary use of antibiotics." According to the results, "POC NAAT would be less costly and more effective than RADT + culture; POC NAAT adoption may yield cost savings to US third-party payers. Access to POC NAAT is important to optimize GAS diagnosis and treatment decisions in the United States" (Bilir et al., 2021).

In a metanalysis, Dubois et al. (2021) studied the diagnostic accuracy of rapid antigen detection tests (NADTs) vs rapid nucleic acid tests (RNATs) for diagnosis of group A streptococcal pharyngitis. 38 studies using RNAT were included, with a sensitivity of 97.5% and specificity of 95.1%. RADTs had a sensitivity of 82.3%, but specificity was similar to the sensitivity of RNATs. Overall, RNATs were more sensitive than

RADTs. The authors conclude that "the high diagnostic accuracy of RNATs may allow their use as stand-alone tests to diagnose group A streptococcus pharyngitis" (Dubois et al., 2021).

V. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

Acute Pharyngitis (CDC, 2022b): Most cases of acute pharyngitis are viral. Only 20-30% of sore throats in children and 5-15% in adults are due to group A *Streptococcus* (GAS). History and clinical examination can be used to diagnosis viral pharyngitis when clear viral symptoms (e.g., cough, rhinorrhea, hoarseness, oral ulcers, conjunctivitis) are present; these patients do not need testing for group A strep. However, clinical examination cannot be used to differentiate viral and group A strep pharyngitis in the absence of viral symptoms, even for experienced clinicians. The diagnosis of group A strep pharyngitis is confirmed by either a rapid antigen detection test (RADT) or a throat culture. RADTs have high specificity for group A strep but varying sensitivities when compared to throat culture, which is considered the gold standard diagnostic test. Testing for group A strep pharyngitis is not routinely indicated for: children younger than 3 years of age or for adults. Clinicians can use a positive RADT as confirmation of group A strep pharyngitis in children, though it also notes that a negative RADT should be followed with a throat culture in children with symptoms of pharyngitis (CDC, 2022b).

The CDC also comments on asymptomatic Group A carriers, stating that these carriers usually do not require treatment. The CDC defines carriers as having “positive throat cultures or are RADT positive, but do not have clinical symptoms or an immunologic response to group A strep antigens on laboratory testing” (CDC, 2022b).

Scarlet Fever (CDC, 2022d): Scarlet fever (scarlatina) consists of an erythematous rash caused by GAS and can occur along with acute pharyngitis. “The differential diagnosis of scarlet fever with pharyngitis includes multiple viral pathogens that can cause acute pharyngitis with a viral exanthema. Clinicians need to use either a rapid antigen detection test (RADT) or throat culture to confirm scarlet fever with pharyngitis. RADTs have high specificity for group A strep but varying sensitivities when compared to throat culture. Throat culture is the gold standard diagnostic test. Clinicians should follow up a negative RADT in a child with symptoms of scarlet fever with a throat culture. Clinicians should have a mechanism in place to contact the family and initiate antibiotics if the back-up throat culture is positive” (CDC, 2022d).

Post-Streptococcal Glomerulonephritis (PSGN) (CDC, 2022d): PSGN is primarily due to a GAS infection, but rare cases of GCS-induced PSGN have been reported. Clinical features include edema, hypertension, proteinuria, macroscopic hematuria, and lethargy. As such, “The differential diagnosis of PSGN includes other infectious and non-infectious causes of acute glomerulonephritis. Clinical history and findings with evidence of a preceding group A strep infection should inform a PSGN diagnosis. Evidence of preceding group A strep infection can include isolation of group A strep from throat or skin lesions or elevated streptococcal antibodies” (CDC, 2022c).

Acute Rheumatic Fever (CDC, 2022a): “The differential diagnosis of acute rheumatic fever is broad due to the various symptoms of the disease. The differential diagnosis may include but is not limited to:

rheumatoid arthritis, juvenile idiopathic arthritis, septic arthritis, systemic lupus erythematosus, serum sickness, Lyme disease, infective endocarditis, viral myocarditis, Henoch-Schonlein purpura, gout, sarcoidosis, leukemia, and Hodgkin's disease." The CDC notes that no definitive diagnostic test exists for acute rheumatic fever and recommends using the Jones criteria (endorsed by the American Heart Association) to make a clinical diagnosis, which "n ow includes the addition of subclinical carditis as a major criteria and stratification of the major and minor criteria based upon epidemiologic risk (e.g., low, moderate, or high risk populations)" (CDC, 2022a).

American Association of Pediatrics (AAP)

The AAP has published the Red Book (Kimberlin et al., 2021) as guidance for infectious diseases in the pediatric population. Their relevant comments and recommendations include:

- "Children with pharyngitis and obvious viral symptoms (eg, rhinorrhea, cough, hoarseness, oral ulcers) should not be tested or treated for GAS [Group A Streptococcus] infection; testing also generally is not recommended for children younger than 3 years."
- "Several rapid diagnostic tests for GAS pharyngitis are available…Specificities of these tests generally are high (very few false-positive results), but the reported sensitivities vary considerably (ie, false-negative results occur)."
- "The US Food and Drug Administration (FDA) has cleared a variety of rapid tests for use in home settings. Parents should be informed that home use is discouraged because of the risk of false-positive testing that represents colonization."
- "Because of the very high specificity of rapid tests, a positive test result does not require throat culture confirmation. Rapid diagnostic tests using techniques such as polymerase chain reaction (PCR), chemiluminescent DNA probes, and isothermal nucleic acid amplification tests have been developed…Some studies suggest that these tests may be as sensitive as standard throat cultures on sheep blood agar."
- "Children with manifestations highly suggestive of viral infection, such as coryza, conjunctivitis, hoarseness, cough, anterior stomatitis, discrete ulcerative oral lesions, or diarrhea, are very unlikely to have true GAS pharyngitis and should not be tested."
- "Testing children younger than 3 years generally is not indicated. Although small outbreaks of GAS pharyngitis have been reported in young children in child care settings, the risk of ARF is so remote in young children in industrialized countries that diagnostic studies for GAS pharyngitis generally are not indicated for children younger than 3 years."
- "In contrast, children with acute onset of sore throat and clinical signs and symptoms such as pharyngeal exudate, pain on swallowing, fever, and enlarged tender anterior cervical lymph nodes, without concurrent viral symptoms and/or exposure to a person with GAS pharyngitis, are more likely to have GAS infection and should have a rapid antigen test and a throat culture if the rapid test result is negative, with treatment initiated if a test result is positive."
- "Testing asymptomatic household contacts for GAS infection is not recommended except when the contacts are at increased risk of developing sequelae of GAS infection, such as ARF or acute glomerulonephritis; if test results are positive, such contacts should be treated."

β-Hemolytic Streptococcus Testing, continued

- "Testing asymptomatic household contacts usually is not helpful. However, if multiple household members have pharyngitis or other GAS infections, simultaneous cultures of all household members and treatment of all with positive cultures or rapid antigen test results may be of value."
- "In suspected invasive GAS infections, cultures of blood and of focal sites of possible infection are indicated."
- "Laboratory evidence of antecedent GAS infection should be confirmed in all cases of suspected ARF [acute rheumatic fever], and evidence includes an increased or rising ASO or anti-DNAase B titer, or a positive rapid antigen or streptococcal throat culture. Because of the long latency between GAS infection and presentation with chorea, such laboratory evidence may be lacking in cases where chorea is the major criteria."
- "Post-treatment throat swab cultures are indicated only for patients who are at particularly high risk of ARF [acute rheumatic fever] (eg, those living in an area with endemic infection)."

Regarding the management of infants at risk of group B streptococcal disease, a list of recommendations was provided. The relevant points are included below:

- "Early-onset GBS infection is diagnosed by blood or CSF culture. Common laboratory tests such as the complete blood cell count and C-reactive protein do not perform well in predicting early-onset infection, particularly among well-appearing infants at lowest baseline risk of infection."
- "Evaluation for late-onset GBS disease should be based on clinical signs of illness in the infant. Diagnosis is based on the isolation of group B streptococci from blood, CSF, or other normally sterile sites. Late-onset GBS disease occurs among infants born to mothers who had positive GBS screen results as well as those who had negative screen results during pregnancy. Adequate IAP does not protect infants from late-onset GBS disease" (Puopolo et al., 2019).

American Heart Association (AHA)

The AHA published a revision to the Jones criteria for diagnosis of acute rheumatic fever in 2015. In it, they note the importance of identifying laboratory evidence of a group A streptococcal infection. The AHA lists three clinical features that can serve as evidence for a preceding Group A Streptococcus infection, which are as follows:

- "Increased or rising anti-streptolysin O titer or other streptococcal antibodies (anti-DNASE B). A rise in titer is better evidence than a single titer result."
- "A positive throat culture for group A β-hemolytic streptococci."
- "A positive rapid group A streptococcal carbohydrate antigen test in a child whose clinical presentation suggests a high pretest probability of streptococcal pharyngitis." (Gewitz et al., 2015)

2017 Institute for Clinical Systems Improvement (ICSI)

In 2017, the ICSI updated their guidelines titled *Diagnosis and treatment of respiratory illness in children and adults*. They give the following consensus recommendation: "It is the consensus of the ICSI work group to NOT test for Group A Streptococcal (GAS) pharyngitis in patients with modified Centor criteria scores less than three or when viral features like rhinorrhea, cough, oral ulcers and/or hoarseness are

present. Testing should generally be reserved for patients when there is a high suspicion for GAS and for whom there is intention to treat with antibiotics (Short et al., 2017)." The Centor criteria include age of patient, physical state of the tonsils and lymph nodes, temperature, and presence or absence of cough (Centor & McIsaac, 2022).

American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA)

The ATS and IDSA published a joint guideline on the diagnosis and treatment of community-acquired pneumonia in adults. The guideline notes that group A *Streptococcus* may be associated with influenza pneumonia. Their relevant recommendations are listed below:

- "We recommend not obtaining sputum Gram stain and culture routinely in adults with CAP managed in the outpatient setting (strong recommendation, very low quality of evidence)."
- "We recommend not obtaining blood cultures in adults with CAP managed in the outpatient setting (strong recommendation, very low quality of evidence)." (Metlay et al., 2019)

Infectious Diseases Society of America (IDSA)

The 2014 update of the IDSA's guidelines concerning skin and soft tissue infections included a recommendation (strong; moderate-quality evidence) of "Gram stain and culture of the pus or exudates from skin lesions of impetigo and ecthyma are recommended to help identify whether *Staphylococcus aureus* and/or β-hemolytic *Streptococcus* is the cause, but treatment without these studies is reasonable in typical cases." They make a similar recommendation in the cases of pus from carbuncles and abscesses as well as pyomyositis; however, they do not recommend (strong, moderate) a "Gram stain and culture of pus from inflamed epidermoid cysts". As for erysipelas and cellulitis, "cultures of blood or cutaneious aspirates, biopsies, or swabs are not routinely recommended (strong, moderate) …cultures of blood are recommended (strong, moderate), and cultures and microscopic examination of cutaneious aspirates, biopsies, or swabs should be considered in patients with malignancy on chemotherapy, neutropenia, severe cell-mediated immunodeficiency, immersion injuries, and animal bites (weak, moderate)." (Stevens et al., 2014)

IDSA and the American Society for Microbiology (ASM) published a guideline in 2018 titled "A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases". This guideline includes items on the laboratory diagnosis of pharyngitis, which are as follows:

- For *Streptococcus pyogenes*, direct NAAT, nucleic acid probe tests, or a rapid direct antigen test (followed by a culture or NAAT test if negative) may all be performed.
- For Groups C and G β-hemolytic streptococci, a NAAT may be performed, or a combination of throat culture and antigen tests on isolates for groups C and G streptococci may be performed.

Other relevant comments include:

- "A rapid antigen test for *Streptococcus pyogenes* may be performed at the point of care by healthcare personnel or transported to the laboratory for performance of the test…in pediatric patients, if the direct antigen test is negative, and if the direct antigen test is known to have a sensitivity of <80%, a second throat swab should be examined by a more sensitive direct NAAT or by

culture as a means of arbitrating possible false-negative direct antigen test results…this secondary testing is not necessarily required in adults"

- "Direct and amplified NAATs for *Streptococcus pyogenes* are more sensitive than direct antigen tests and, as a result, negative direct NAAT results do not have to be arbitrated by a secondary test."
- "Detection of group C and G β-hemolytic streptococci is accomplished by throat culture in those patients in whom there exists a concern for an etiologic role for these organisms. Only large colony types are identified, as tiny colonies demonstrating groups C and G antigens are in the *Streptococcus anginosus (S. milleri)* group." (Miller et al., 2018)

American Academy of Otolaryngology-Head and Neck Surgery Foundation

Although the main focus of this guideline is the tonsillectomy procedure in children, there are some relevant comments. The Academy notes that "In practice, streptococcal carriage is strongly suggested by positive strep cultures or other strep tests when the child lacks signs or symptoms of acute pharyngitis." (Mitchell et al., 2019) IDSA endorsed this guideline in February 2019 (IDSA, 2019a).

American Academy of Orthopaedic Surgeons

Although this guideline focuses on management of periprosthetic joint infections, there is a relevant recommendation, which states that "synovial fluid aerobic and anaerobic bacterial cultures" have moderate evidence to support their use to "aid in the diagnosis of prosthetic joint infection (PJI)" (AAOS, 2019). IDSA endorsed this guideline in March 2019 (IDSA, 2019b).

2011 Pediatric Infectious Diseases Society (PIDS) and Infectious Diseases Society of America (IDSA)

The 2011 joint PIDS-IDSA guidelines concerning pediatric community-acquired pneumonia (CAP) recommended (strong recommendation; moderate-quality evidence) that "blood cultures should not be routinely performed in nontoxic, fully immunized children with CAP managed in the outpatient setting" and that "blood cultures should be obtained in children who fail to demonstrate clinical improvement and in those who have progressive symptoms or clinical deterioration after initiation of antibiotic therapy". Concerning inpatient services, they recommend (strong recommendation; low-quality evidence) that "blood cultures should be obtained in children requiring hospitalization for presumed bacterial CAP that is moderate to severe, particularly those with complicated pneumonia"; however, "in improving patients who otherwise meet criteria for discharge, a positive blood culture with identification or susceptibility results pending should not be routinely preclude discharge of that patient with appropriate oral or intravenous antimicrobial therapy. The patient can be discharged if close follow-up is assured (weak recommendation; low-quality evidence)". For pneumococcal bacteremia, they do not recommend repeated blood cultures to document resolution (weak recommendation; low-quality evidence), but they do recommend "repeated blood cultures to document resolution of bacteremia…caused by *S. aureus*, regardless of clinical status (strong recommendation; low-quality evidence)". With respect to sputum gram stain and culture, "sputum samples for culture and Gram stain should be obtained in hospitalized children who can produce sputum" (weak recommendation; low-quality evidence). They do not recommend using urinary antigen detection testing "for the diagnosis of pneumococcal pneumonia in

children; false-positive tests are common (strong recommendation; high-quality evidence) (Bradley et al., 2011)."

American College of Obstetricians and Gynecologists

The ACOG issued Committee Opinion #797 in 2020. ACOG recommends that "Regardless of planned mode of birth, 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" (ACOG, 2020).

American Society for Microbiology

The ASM endorsed the above ACOG recommendation, stating that "The recommended screening interval has changed from 35-37 weeks (per CDC 2010 guidelines) to 36 0/7 to 37 6/7 weeks (ACOG 2019 recommendations)". Concerning identification of group B *streptococcus*, the ASM propounds the following:

"Recommendation: Acceptable phenotypic and proteomic methods of identification of candidate isolates include CAMP test, latex agglutination, and mass spectrometry."

"Recommendation: Nucleic acid amplification-based identification of GBS from enrichment broth is acceptable, but not sufficient for all patients."

"Recommendation: Latex agglutination directly from enrichment broth and direct-from-specimen immunoassays are unacceptable methods for GBS detection."

The guideline also recommends performing "antimicrobial susceptibility testing on all GBS [Group B Streptococcus] isolates from pregnant women with penicillin allergy", and most recently the ASM included options for vancomycin reporting (Filkins et al., 2021).

National Institute for Health and Care Excellence

NICE published an update on "rapid tests for group A streptococcal infections in people with a sore throat". They stated that "Rapid tests for strep A infections are not recommended for routine adoption for people with a sore throat. This is because their effect on improving antimicrobial prescribing and stewardship, and on patient outcomes, as compared with clinical scoring tools alone, is likely to be limited." (NICE, 2019)

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA approved the Lyra Direct Strep Assay (k133833) on 04/16/2014 and reclassified it on 07/11/2014. It is a "Real-Time PCR *in vitro* diagnostic test for the qualitative detection and differentiation of Group A β -hemolytic *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C and G β -hemolytic *Streptococcus* nucleic acids isolated from throat swab specimens obtained from

patients with signs and symptoms of pharyngitis, such as sore throat. The assay does not differentiate between pyogenic Groups C and G β-hemolytic *Streptococcus* (Hojvat, 2014)." The FDA has also approved the Solana Strep Complete Assay by Quidel that is "an in vitro diagnostic test for the detection of Group A, C and G beta- hemolytic *Streptococcus* in throat swab specimens from symptomatic patients" on 10/25/2016 (K162274) (FDA, 2016).

On 03/06/2019, the FDA approved GenePOC's Strep A assay to be performed using GenePOC's Revogene instrument as a "single-use test for qualitative detection of *Streptococcus pyogenes* (group A *Streptococcus*-GAS) nucleic acids from throat swab specimens obtained from patients with signs and symptoms of pharyngitis (FDA, 2019)."

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
83789	Mass spectrometry and tandem mass spectrometry (eg, MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
86060	Antistreptolysin 0; titer
86063	Antistreptolysin 0; screen
86215	Deoxyribonuclease, antibody
86317	Immunoassay for infectious agent antibody, quantitative, not otherwise specified
86318	Immunoassay for infectious agent antibody, qualitative or semiquantitative, single step method (eg, reagent strip)
87040	Culture, bacterial; blood, aerobic, with isolation and presumptive identification of isolates (includes anaerobic culture, if appropriate)
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87071	Culture, bacterial; quantitative, aerobic with isolation and presumptive identification of isolates, any source except urine, blood or stool
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87081	Culture, presumptive, pathogenic organisms, screening only
87430	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; Streptococcus, group A
87650	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group A, direct probe technique

87651	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group A, amplified probe technique
87652	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group A, quantification
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87880	Infectious agent antigen detection by immunoassay with direct optical (ie visual) observation; Trichomonas vaginalis

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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Allergen Testing

Policy #: AHS – G2031	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/26/22, 5/20/22 (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

Allergic disease is characterized by inappropriate or exaggerated immune reactions to foreign antigens (allergens) that are generally innocuous to most people, but when introduced into a genetically-predisposed individual, elicit a hypersensitivity reaction (R. Hamilton, 2020). Hypersensitivity reactions can be classified into four types, two of which are associated with allergy, type I immediate immunoglobulin E (IgE) reactions and type IV T cell mediated reactions (K.-L. Chang & J. C. Guarderas, 2018). Type I reactions involve the formation of IgE antibodies specific to the allergen. When the subject is re-exposed to that allergen, the allergen binds multiple IgE molecules, resulting in the release of an array of inflammatory mediators, including histamines, that precipitate the symptoms of allergic disease (R. Hamilton, 2018).

Allergen testing in serum is designed to detect the presence of allergen-specific IgE. A positive test for allergen-specific IgE confirms the presence of the antibody only. Actual reactivity must be determined by history or supervised challenge (Kowal & DuBuske, 2020). Several diagnostic procedures have been developed to elicit and assess hypersensitivity reactions including epicutaneous, intradermal, patch, bronchial, exercise, and ingestion challenge tests (Bernstein et al., 2008).

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.

Allergen Testing, continued

1. Specific IgE in-vitro allergy testing **MEETS COVERAGE CRITERIA** in any of the following situations:
   a. In lieu of skin testing for an initial allergy screen. When in-vitro testing is ordered, the medical record must clearly document the indication and why it is being used instead of skin testing.
   b. When skin testing is either contraindicated (see Note 1)
   c. When further treatment decisions would be impacted by confirmation of sensitivity in individuals for whom direct skin testing results are not consistent with the history of an anaphylactic or other severe reaction to an allergen.
2. When limited to allergens chosen for testing based on the individual's history, physical examination, and environment, specific IgE in-vitro allergy testing (up to 30 allergen specific antibodies per year) **MEETS COVERAGE CRITERIA**.
3. In-vitro testing for total serum IgE **MEETS COVERAGE CRITERIA** in any of the following situations:
   a. For individuals with moderate to severe asthma being considered for Xolair therapy.
   b. For individuals who are suspected of having allergic bronchopulmonary aspergillosis.
4. To monitor for allergy resolution in children and adolescents with an initial positive food allergen result(s), annual re-testing for the same food allergen(s) **MEETS COVERAGE CRITERIA**.
5. In the absence of a new clinical presentation, routine re-testing for allergies to the same allergens (except where specified above) **DOES NOT MEET COVERAGE CRITERIA**.
6. The Antigen Leukocyte Antibody test (ALCAT) **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. For the evaluation of a suspected allergy, in-vitro testing of IgG, IgA, IgM, and/or IgD **DOES NOT MEET COVERAGE CRITERIA**.
8. To measure hypersensitivity to allergens, basophil activation flow cytometry testing (BAT) **DOES NOT MEET COVERAGE CRITERIA.**
9. In-vitro allergen testing using bead-based epitope assays (e.g., VeriMAP Peanut Dx) **DOES NOT MEET COVERAGE CRITERIA.**
10. For all situations, in-vitro testing using qualitative specific IgE multi-allergen screen that does not identify a specific allergen **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Skin testing is contraindicated in the following situations:

- Patients who have certain skin conditions (e.g., dermatographism, urticaria, cutaneous mastocytosis

atopic dermatitis, severe diffuse psoriasis)

- Patients who are taking medications that may interfere with the treatment of anaphylaxis (e.g., Beta-blockers and Angiotensin Converting Enzyme inhibitors) or may impair skin test sensitivity (e.g., tricyclic antidepressants, antihistamines)
- Patients who are at high risk to testing (e.g., poorly controlled asthma, clinical history of severe reaction to minute amounts of allergen, cardiac arrhythmia, unstable angina)
- Patients who have experienced an anaphylactic event within the past one month
- Uncooperative patients (e.g., small children, individuals with mental or physical impairments)

III. Scientific Background

Allergies affect over 50 million Americans, including approximately 30 percent of adults and 40 percent of children (Jackson et al., 2013; NASEM, 2016). The incidence of allergic disease is increasing (Pawankar et al., 2013) and is estimated to result in over $17 billion in health care costs and 200,000 emergency department visits annually (Adams et al., 2013).

A majority of environmental, food, and medication allergies with clinical significance are type I immunoglobulin E (IgE)-mediated allergies (Kowal & DuBuske, 2022). Diagnosis of an IgE-mediated allergy involves identification of the allergen, demonstration of IgE specific to that allergen, and confirmation that symptoms occur when the patient is exposed to the allergen. The IgE response to an allergen can be assessed using skin or serum testing. Patch testing is preferred for delayed T-cell mediated response (K.-L. Chang & J. C. Guarderas, 2018; Zug et al., 2014).

Allergic diseases, respiratory infections, and autoimmune conditions have similar clinical presentations and self-reported symptoms have a relatively low positive predictive value (PPV) (Sampson et al., 2014). Thus, laboratory allergy and immunologic testing are useful in clarifying diagnosis and guiding treatment when the frequency, duration, and sequelae of upper respiratory infections exceed the norm or when rhinosinusitis or asthma symptoms persist despite treatment (Chow et al., 2012). Allergy testing is also useful in identifying causative allergen in atopic dermatitis (eczema), contact dermatitis, urticaria, angioedema, and food or drug allergies. Knowing the causal allergen helps provide clinically relevant information for avoidance and treatment (K.-L. Chang & J. C. Guarderas, 2018).

Skin Testing

Skin testing is the most rapid, sensitive, and cost-effective testing modality for the detection of immunoglobulin E (IgE)-mediated disease. The procedure lasts less than an hour with minimal patient discomfort. There are several published practice parameters for allergen skin testing (Bernstein et al., 2008; K.-L. Chang & J. C. Guarderas, 2018; Kowal & DuBuske, 2022).

Serum IgE

IgE is one of five immunoglobulins and the one primarily involved in allergic disease. At the cellular level, the allergic response starts with “atopy,” a genetic predisposition to produce specific IgE after exposure to allergens. CD4+ helper T cells are predisposed to the “T helper type 2” (Th2) response, which causes

the Th2 cells to secrete large amounts of interleukins 4 and 13, which then promotes production of the allergen-specific IgE. From there, the allergen-specific IgE binds to high-affinity receptors on mast cells and basophils. At this point, if the relevant allergen is ingested in large enough amounts, the IgE molecules may cluster (cross-linking). This cross-linking causes the mast cells and basophils to release chemical and protein mediators, resulting in the characteristic allergic response (Stokes & Casale, 2019).

Immunoassays measuring both total IgE and allergen-specific IgE in serum and other bodily fluids have been developed. Specific IgE immunoassays do not require patient cooperation, are not limited in patients with skin disease, are not blocked by antihistamines, and pose no risk of adverse reactions (Bernstein et al., 2008; K.-L. Chang & J. C. Guarderas, 2018; Stokes & Casale, 2019). Total IgE is usually unrelated to IgE levels for a specific allergen but may be useful in other conditions, such as asthma (Stokes & Casale, 2022).

Other Testing

Patch testing is the gold standard for identification of a contact allergen (Mowad, 2006; Rietschel, 1997). Although occlusive patch testing is the most common technique, open, prophetic (provocative), repeated insult, photopatch, and atopy patch tests are also available if special situations indicate their use (Bernstein et al., 2008).

Cellular activation assays measuring the release of histamine from basophils (Kim et al., 2016; Santos & Lack, 2016) or mast cells (Bahri et al., 2018) as diagnostic or prognostic indicators of allergy have been the subject of intense research. Basophil and eosinophilic reactivity tests have been found to be associated with food-induced allergic responses and have been shown in current research to be modified over time during immunotherapy (Sampson et al., 2014). The basophil activation test (BAT) in particular has emerged as having superior specificity and comparable sensitivity to diagnose food allergies when compared with skin prick test and specific IgE (Santos & Shreffler, 2017). Histamine release from leukocytes of allergic persons is an excellent in vitro correlate of allergy; however, it is currently still considered a research test by the Academy of Allergy, Asthma, and Immunology (AAAI) (Bernstein et al., 2008).

BAT has the potential to be a useful tool for measuring hypersensitivity to allergens, especially for patients who are not suitable for skin testing due to skin status or prior severe reactions since it is an ex vivo, flow cytometry-based assay. BAT, for use as standard clinical practice, is currently limited by its lack of standardization in methodology as well as between systems used. A study by Depince-Berger et al., (2017) has proposed standardization between systems and instruments using whole blood-ethylenediaminetetraacetic acid (EDTA) samples with instrumentation standardization. “BAT would strongly benefit from easy implementation [EDTA, one step stimulation/labeling, wash, full sample analysis over time parameter, B cell relative basophil count] and standardization of instrument settings on MFI [median fluorescence intensity] targets whatever system or instrument is used” (Depince-Berger et al., 2017). Hemmings et al. (2018) note that standardization, quality assurance, and clinical validation will facilitate the transition of the BAT from research to clinical practice.

Proprietary Testing

The Antigen Leukocyte Antibody Test (ALCAT) is another test available for the assessment of allergens. ALCAT measures food/immune reactions through stimulation of leukocytes. The immunological reactions to this stimulation are intended to identify sensitivities regardless of pathway as antibodies do not necessarily need to be involved. CellScienceSystems suggests individuals with a variety of disorders

(such as gastrointestinal, neurological, et al.) to take this test (CellScienceSystems, 2019). Although the ALCAT machine is FDA registered and there are a few papers published, results are not reproducible when subject to rigorous testing and do not correlate with clinical evidence of allergy (Beyer & Teuber, 2005; Hammond & Lieberman, 2018; Wuthrich, 2005).

Panels encompassing a large number of analytes are also offered by labs. For example, Genova Labs offers a blood test for IgG and IgE antibodies for 87 different foods. Genova also offers several variations on this test, such as "Vegetarian" (21 foods), "Spices" (24 spices), "Molds" (15 molds), and more (Genova, 2019b). A similar test measuring IgG4 antibodies for 90 commonly consumed foods is also offered (Genova, 2019a).

Spiriplex offers a microarray-style panel for allergen testing, called "Allergenex." This test contains many purified allergen proteins to which a patient's blood sample can bind. This binding creates a quantifiable signal that allows the user to identify the number of IgE antibodies present, and therefore provide a picture of allergy. Spiriplex offers a test for 26 common food allergens, a test for 37 inhalant allergens, and 63 combined food and inhalant allergens (Spiriplex, 2017).

The VeriMAP Peanut Dx and the VeriMAP Peanut Sensitivity are both peanut-allergen specific bead-based epitope assays manufactured by AllerGenis LLC. According to Allergenis, VeriMAP has a "95% positive predictive value and can reduce overdiagnosis and anxiety by minimizing false positives" (BioSpace, 2021). This is an emerging technology and additional peer-reviewed literature establishing the analytical validity, clinical validity, and clinical utility of such testing will be further required.

Analytical Validity

Variables that can influence the wheal size when performing skin prick tests (SPT) include multiple operators, extract concentrations and quality, skin test devices, time of day, location on the skin, and the measuring of results (Nelson, 2001; Werther et al., 2012).

In 2006, Oppenheimer and Nelson evaluated variability and analytical validity of skin testing. A questionnaire was sent to all physician and fellow members of the American College of Allergy, Asthma and Immunology who were currently practicing in the United States. The objective of this questionnaire was to determine the diversity of skin testing practices among allergists. The results showed great variability among physicians. In particular, "The average number of skin prick tests performed ranged from 5.09 (grasses) to 10.9 (trees), whereas the average number of intradermal tests performed ranged from 2.03 (grasses) to 5.6 (perennial). The allergen extract concentrations used for intradermal testing varied widely. Expressed as a dilution of the concentrated extracts, 20.8% use 1:100 dilutions, 10.3% use 1:500 dilutions, and 59.4% use 1:1,000 dilutions. Significant variability also occurred regarding devices and the technique with which the devices were used. Most clinicians (92.1%) used the most concentrated extract available for skin prick testing. For reporting the results of skin testing, 53.8% used a 0 to 4+ scale, and only 28.3% measured orthogonal diameters. Of those using a 0 to 4+ scale, two thirds related the results to the size of the histamine control (Oppenheimer & Nelson, 2006)." The results from this survey emphasize potential areas of improvement for allergists regarding skin test use and data.

The Clinical and Laboratory Standards Institute (CLSI) has evaluated the analytical validity of serum IgE measurements and found that "Clinical/diagnostic sensitivity and specificity of IgE antibody assays cannot be accurately determined due to the absence of definitive gold standard methods for defining allergic disease. Total and allergen-specific IgE analyses achieve among the highest analytical

performance of any antibody assay by following consensus procedures in CLSI-ILA20-A3" (R. G. Hamilton et al., 2015).

Knight et al. (2018) "examined the qualitative concordance between SPT and sIgE as measured on the HYTEC™288 platform for 10 commonly encountered inhalant allergens"; a total of 232 subjects were included. Overall concordance between SPT and sIgE was > 70% for all allergens tested. Sensitivity ranged from 25% to 95% depending on the allergen, while specificity was significantly higher for all allergens (78-97%). Negative predictive value (NPV) was > 85% for all allergens tested, while PPV was more variable, ranging from 22% to 88%. The authors noted that "these results are similar to findings in other studies comparing SPT with sIgE" (Knight et al., 2018).

Carlsson et al. (2015) examined the inter- and intra- variability of IgE and IgE receptor expression in the blood of seasonal allergic rhinitis (SAR) subjects. Thirty-two patients with SAR were included; the high-affinity IgE receptor, also known as FcεRI, and the low affinity receptor, also known as CD23, were measured. The authors found that "FcεRI expression on basophils and CD23 expression on B cells showed low intrasubject variability both in and out of the pollen season," although there was a small seasonal difference with lower total IgE levels and FcεRI expression during the pollen season (Carlsson et al., 2015).

Siroux et al. (2017) explored the effect of allergen nature, route of exposure, and dose of exposure on IgE and IgG responses. A total of 340 patients (170 with asthma, 170 without) were included, and IgE/IgG responses to 47 inhalant and food allergens were analyzed and compared between 5 French regions according to route of allergen exposure (inhaled or food). "Ubiquitous" allergens (grass, olive/ash pollen, house dust mites) did not show marked difference in specific IgE level between regions. For region-specific allergens (ragweed, birch, cypress), IgE sensitization was associated with regional pollen exposure. Airborne allergens cross-reacting with food allergens led to frequent IgG recognition. The authors concluded that "the variability in allergen-specific IgE and IgG frequencies depends on exposure, route of exposure, and overall immunogenicity of the allergen. Allergen contact by the oral route might preferentially induce IgG responses" (Siroux et al., 2017).

Sookrung et al. (2019) measured the agreement of a SPT and serum-specific IgE test to Periplaneta americana (American cockroach, ACR) allergies. ACR-extract was used, and sera was obtained from 66 individuals clinically diagnosed with chronic allergic rhinitis. Of the 66 samples, 46 were positive and 20 negative after a SPT to ACR-extract. Serum IgE levels were then measured by a commercial test kit. The authors note that of the SPT positive cases to ACR-extract, only 32.6% were also positive for serum IgE, indicating low concordance between the two testing methods (Sookrung et al., 2019).

He and Reisacher (2019) measured the sensitivity, specificity, and predictive value of oral mucosal brush biopsies (MBB) as a new diagnostic test for peanut allergies. Twenty individuals participated in this study; each participant underwent oral MBB and serum testing for peanut IgE. The authors note that "At 0.12 kU/L, the sensitivity of oral MBB testing was 80% and the specificity was 85%, whereas at 1.0 kU/L, the sensitivity of sIgE testing was 50% and the specificity was 100%. From the ROC curves, the areas under the ROC curve (AUC) for oral MBB and sIgE were 0.91 ($p < 0.001$) and 0.74 ($p = 0.007$), respectively. Combination testing further increased both sensitivity and accuracy over oral MBB alone" (He & Reisacher, 2019). These results are promising for oral MBB, although more research needs to be completed.

Clinical Utility and Validity

Allergen Testing, continued

In 1998, Tschopp et al. (1998) compared three diagnostic tests for atopic diseases. Total serum IgE, Phadiatop, and the SPT were compared for 8329 individuals. Current allergic asthma (CAA) and current allergic rhinitis (CAR) were the conditions studied. The prevalence of CAA was 1.8% and prevalence for CAR was 16.3%. The prevalence of positive tests was 29%, 23%, and 23% for Phadiatop, SPT, and IgE, respectively. The results were as follows: "To diagnose current allergic asthma (CAA) and current allergic rhinitis (CAR), the sensitivity of Phadiatop was significantly higher than that of SPT (72.5% vs 65.4%, 77.1% vs 68.4% respectively) and IgE (72.5% vs 56.9%, 77.1% vs 43.9%, respectively. The sensitivity of SPT was significantly higher (68.4% vs 43.9%) than that of IgE to diagnose CAR. When CAA and CAR were excluded, the SPT specificity was significantly higher than that of Phadiatop (77.8% vs 71.9% and 85.9% vs 80.5%, respectively): when CAR was excluded, SPT was significantly higher than IgE (85.9 vs 81.4%). SPT had significantly the best positive predictive value for CAA (5.2% for SPT vs 4.6% for both IgE and Phadiatop) and CAR (48.7% for SPT vs 43.5% for Phadiatop and 31.6% for IgE). The three markers of atopy had roughly the same negative predictive value (NPV) for CAA, but IgE had a significantly lower NPV for CAR than SPT and Phadiatop (88.1% vs 93.3% and 94.7%, respectively). The diagnostic efficiency of SPT was significantly higher than that of Phadiatop (83.1% vs 79.9% and 77.6 vs 71.9%, respectively) to diagnose CAR and CAA. IgE and SPT had equal efficiency (77.6%), which was significantly higher than that of Phadiatop, to diagnose CAA (71.9%) (Tschopp et al., 1998)." The authors concluded that "SPT have the best positive predictive value and the best efficiency to diagnose respiratory atopic diseases. Furthermore, SPT give information on sensitivity to individual allergens and should therefore be used primarily by clinicians to assess respiratory allergic diseases" (Tschopp et al., 1998).

Usmani and Wilkinson (2007) performed a retrospective analysis of patients who had been prick tested to "establish whether an incomplete diagnosis would have been reached if patch testing had been omitted." The authors observed that if "investigation of allergic skin disease is undertaken by a non-dermatologist, it is unlikely that patch testing will be performed." A total of 330 patients had been prick tested in the time period specified. Sixty-eight patients had positive reactions on prick testing, and 36 of those had positive patch tests. Of the 262 patients who had negative prick tests, 121 had positive patch tests (46.1%) of current relevance to patient history in 92 subjects (35.1%). The authors concluded that "omission of patch testing from the investigation of allergic skin disease, even when contact urticaria may be the sole suspected diagnosis, would result in the frequent missed diagnosis of contact allergy" (Usmani & Wilkinson, 2007).

In 2014, a meta-analysis examined the clinical validity of SPT and IgE measurement for food allergy. Twenty-four studies consisting of 2831 participants were included. The results were as follows: "For cows' milk allergy, the pooled sensitivities were 88% (SPT), and 87% (IgE) and specificities were 68% and 48%. For egg, pooled sensitivities were 92% and 93% and specificities were 58% and 49% for SPT and specific-IgE. For wheat, pooled sensitivities were 73% and 83% and specificities were 73% and 43% for SPT and sIgE. For soy, pooled sensitivities were 55% and 83% and specificities were 68% and 38% for SPT and sIgE. For peanut, pooled sensitivities were 95% and 96%, and specificities were 61% and 59% for SPT and sIgE (Soares-Weiser et al., 2014)."

Klemans et al. (2015) examined the diagnostic accuracy of using sIgE to peanut components to improve sensitivity and specificity of peanut allergen testing. Twenty-two studies were included. The authors found that "sIgE to Ara h 2 [a peanut component] showed the best diagnostic accuracy of all diagnostic tests to diagnose peanut allergy. Compared to the currently used SPT and sIgE to peanut extract, sIgE to Ara h 2 was superior in diagnosing peanut allergy" (Klemans et al., 2015). The authors also found that

Allergen Testing, continued

the worst accuracy was observed to be sIgE to Ara8 and Ara9. The authors concluded that "sIgE to Ara 2 should replace SPT and sIgE to peanut extract in daily clinical practice" (Klemans et al., 2015).

Caglayan Sozmen et al. (2015) examined the diagnostic accuracy of using the patch test to avoid oral food challenge (OFC). They found that in 243 children that underwent OFC to suspected food, clinically relevant food allergies were seen in 40 (65%) children to egg and in 22 (35%) to cow's milk. The sensitivity of SPT for both milk and egg was 92%, specificity 91%, PPV 35%, and NPV 93%. Sensitivity, specificity, PPV, and NPV of atopy patch test for both milk and egg were 21%, 73%, 20%, and 74%, respectively.

Santos et al. (2014) studied the performance of basophil activation tests (BAT) as a diagnostic marker for peanut allergy. Forty-three peanut-allergic children, 36 peanut-sensitized but tolerant children, and 25 non–peanut-sensitized nonallergic children underwent SPT, sIgE, and BAT. The authors found that BAT in peanut-allergic children showed a peanut dose-dependent upregulation of CD63 and CD203c while there was no significant response in the other two cohorts. BAT optimal diagnostic cutoffs showed 97% accuracy, 95% PPV, and 98% NPV. BAT allowed reduction of required oral food challenges (OFCs) by two-thirds. BAT proved particularly useful in cases in which specialists could not accurately diagnose peanut allergy with SPT and sIgE to peanut and to Arah2. Using a 2-step diagnostic approach in which BAT was performed only after equivocal SPT or Arah2-sIgE, BAT had a major effect (97% reduction) on the number of OFCs required.

Santos et al. (2015) also studied the utility of BAT to predict the severity and reactivity to peanut during OFCs. They found that "Of the 124 children submitted to OFCs to peanut, 52 reacted with clinical symptoms that ranged from mild oral symptoms to anaphylaxis. Severe reactions occurred in 41% of cases, and 57% reacted to 0.1 g or less of peanut protein. The ratio of the percentage of CD63(+) basophils after stimulation with peanut and after stimulation with anti-IgE (CD63 peanut/anti-IgE) was independently associated with severity, whereas the basophil allergen threshold sensitivity CD-sens ($1/EC_{50} \times 100$, where EC_{50} = half maximal effective concentration) value was independently associated with the threshold of allergic reactions to peanut during OFCs. Patients with CD63 peanut/anti-IgE levels of 1.3 or greater had an increased risk of severe reactions (relative risk, 3.4). Patients with a CD-sens value of 84 or greater had an increased risk of reacting to 0.1 g or less of peanut protein (relative risk, 1.9) (Santos et al., 2015)." The authors concluded that "Basophil reactivity is associated with severity, and basophil sensitivity is associated with the threshold of allergic reactions to peanut. CD63 peanut/anti-IgE and CD-sens values can be used to estimate the severity and threshold of allergic reactions during OFCs" (Santos et al., 2015).

Davila et al. (2015) explored the association between total IgE and severity of asthma. A total of 383 patients were included (129 mild, 82 moderate, and 172 severe). Serum IgE levels were noted to vary "markedly" (147% coefficient of variation). The authors did not find an association between total IgE and forced expiratory volume in 1 second (FEV1) or asthma severity; although, the severe subgroup had a higher percentage of patients with >400 IU/mL. Independent predictors of higher IgE were found to be younger age, sensitization to ≥ 2 allergens, male gender, and family history of asthma. The authors concluded that "we did not find a significant association between serum total IgE levels and asthma severity or airflow limitation, except for a higher percentage of patients with IgE > 400 IU/mL in the severe subgroup" (Davila et al., 2015).

Tannert et al. (2017) investigated the relevance of a positive skin test and positive IgE test to penicillin allergy. Twenty-five patients with positive results were given penicillin, and another 19 patients deemed

allergic were included. However, only 9 of the 25 patients given penicillin were challenge-positive. Positive results from each test alone did not predict allergy. The authors concluded that "the best predictor for a clinically significant (IgE-mediated) penicillin allergy is a combination of a positive case history with simultaneous positive ST result and s-IgE or a positive challenge result" (Tannert et al., 2017).

Suárez-Fariñas et al. (2021) investigated the validity of the peanut BBEA diagnostic test on 133 subjects as well as on 82 additional subjects from another study, forming a cohort for a paper titled, "Accurate and reproducible diagnosis of peanut allergy using epitope mapping." The authors measured levels of IgE to epitopes evaluated against a threshold established prior to the study. The peanut BBEA diagnostic test diagnosed 93% of subjects accurately, with a sensitivity threshold of 92% and specificity of 94%. The positive predictive value (PPV) was 91%. The authors concluded that "the overall accuracy was found to be superior to existing diagnostic tests for peanut allergy including skin prick testing, peanut sIgE, and peanut component sIgE testing" (Suárez-Fariñas et al., 2021).

IV. Guidelines and Recommendations

The American Academy of Allergy, Asthma and Immunology (AAAAI) and the American College of Allergy, Asthma and Immunology (ACAAI)

The AAAI and ACAAI published practice parameters in 2008 for allergy testing (Bernstein et al., 2008) which noted that "For individual patients, the choice of test allergens is guided by the history and physical examination and the physician's knowledge, training, and experience." The guidelines recommended that "Specific IgE immunoassays may be preferable to skin testing under special clinical conditions, such as widespread skin disease, patients receiving skin test suppressive therapy, uncooperative patients, or when the history suggests an unusually greater risk of anaphylaxis from skin testing." They also note that for both skin testing and in-vitro specific IgE testing, "the allergens selected … should be determined based on the patient's age, history, environment and living conditions (e.g., region of the country), occupation, and activities." Also, "The best indicators in the selection of appropriate pollens for clinical use are extensive prevalence in the air and concurrent allergy symptoms during annually recurrent seasons when such pollens are expected to be present in the ambient air."

They AAAAI and ACAAI guidelines also state, "As is the case with skin tests, a direct correlation cannot be assumed between the presence of specific IgE (sIgE) antibodies and clinical disease." Additionally, "sensitivity and the positive predictive value of both prick/puncture and specific IgE tests generally tend to be higher among pollens, stable anaphylactogenic foods, house dust mite, certain epidermals, and fungi compared with venoms, drugs, and chemicals."

With regards to total IgE testing, these groups indicate, "Measurements of total serum IgE concentration are of modest clinical value when used as a screen for allergic disease or for predicting the risk of allergic disease."

The AAAAI and ACAAI also note that "IgG and IgG subclass antibody tests for food allergy do not have clinical relevance, are not validated, lack sufficient quality control, and should not be performed."

With regards to basophil activation assays they state, "Histamine and leukotriene release measurements from human basophils after incubation with allergen are valuable research tools for in vitro investigations of allergy (Bernstein et al., 2008)."

Allergen Testing, continued

Their practice parameter on drug allergy also states that "The basophil activation test is a recently described method of evaluating expression of CD63 on basophils after stimulation with an allergen. There are limited data using this method to evaluate patients with possible allergies to β-lactam antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs)" (Boyce et al., 2010).

They also recommend, "Because anaphylactic reactions cannot be distinguished from anaphylactoid, nonimmune occurrences, it has been recommended that plasma histamine, tryptase, and specific IgEs (if available) may be ordered at the time of reaction and skin tests be performed later" (Boyce et al., 2010).

In their 2014 practice parameter on food allergy (Sampson et al., 2014) they acknowledge: "Basophil and eosinophilic reactivity tests have been shown to be associated with food-induced allergic responses and have been shown in current research to be modified over time during immunotherapy."

Their 2014 practice parameter on rhinosinusitis also recommends to "Perform an evaluation for specific IgE antibodies to airborne allergens in patients with RARS or CRS." An updated practice parameter on rhinitis published in 2020 comments that local allergic rhinitis will often be associated with negative skin prick tests (and intradermal tests, when performed) and absence of serum-specific IgE (sIgE) antibodies but a positive nasal allergen provocation test (NAPT) to aeroallergens (Dykewicz et al., 2020). With respect to vasomotor rhinitis, the authors state that "… laboratory tests, skin prick tests, and sIgE are helpful only to exclude AR [allergic rhinitis]."

In this practice parameter, they also make the following summary concerning re-evaluation of food allergies in children and adolescents: "Summary Statement 11: Consider the natural course of allergies to specific foods when deciding on the frequency of food allergy follow-up evaluations, recognizing that allergies to certain foods (milk, egg, wheat, and soy) generally resolve more quickly in childhood than others (peanut, tree nuts, fish, and shellfish). These observations could support individualized follow-up (ie, roughly yearly re-evaluations of these allergies in childhood) with less frequent retesting if results remain particularly high (eg, >20-50 kUA/L). [Strength of recommendation: Moderate; C Evidence]."

In their 2015 practice parameter on anaphylaxis (Lieberman et al., 2015), they recommend "Skin tests and/or in vitro tests for specific IgE and challenge tests might be appropriate to help define the cause of the anaphylaxis."

They also recommend against routinely obtaining total serum IgE levels for the diagnosis of food allergy, however because of the low PPV of self-reported symptoms and lack of pathognomonic signs on physical examination, they recommend that the accurate diagnosis of IgE-mediated food allergy should be aided by laboratory allergy testing, including skin prick and/or serum IgE testing. The clinician should use specific IgE tests (skin prick tests, serum tests, or both) to foods as diagnostic tools; however, testing should be focused on foods suspected of provoking the reaction, and test results alone should not be considered diagnostic of food allergy. Moreover, "The diagnosis of food-induced anaphylaxis should be based on signs and symptoms in association with likely or known exposure to a food allergen", as "Events mimicking anaphylaxis also can occur after the ingestion of food" (Lieberman et al., 2015).

In a Choosing Wisely (CW) report, the AAAAI recommends against performing "unproven diagnostic tests, such as immunoglobulin G (lgG) testing or an indiscriminate battery of immunoglobulin E(lgE) tests, in the evaluation of allergy" (AAAAI, 2012).

In another CW report, the AAAAI recommends against routine diagnostic testing in patients with chronic urticaria, stating that "skin or serum-specific IgE testing for inhalants or foods is not indicated, unless

there is a clear history implicating an allergen as a provoking or perpetuating factor for urticaria" (AAAAI, 2012).

The AAAAI also published a 2020 practice parameter update on peanut allergy diagnosis. The authors recommend in favor of diagnostic skin prick test or sIgE testing for peanut allergy in patients with physician-judged high pretest probability of peanut allergy. Testing is also recommended prior to an oral food challenge for patients with moderate pretest probability of peanut allergy. Ara h 2 diagnostic testing is the suggested approach for patients presenting for evaluation of suspected peanut allergy for which a single diagnostic test is to be used, due to its superior diagnostic accuracy "by virtue of more optimal positive/negative likelihood ratios." However, Ara h 2 is noted to have lower sensitivity than the skin prick or sIgE tests, so a clinician may use Ara h 2, SPT, or sIgE to confirm the diagnosis of peanut allergy in a patient with a high prior probability. The AAAAI recommends against "routine use of component testing in addition to either SPT or sIgE to whole peanut to increase diagnostic accuracy", and also against using the results of skin prick or sIgE to determine "the severity of an allergy phenotype or to predict the severity of a future reaction."

It is noteworthy that all of the recommendations above were assigned "low" or "very low" degrees of evidence certainty (Greenhawt et al., 2020).

Joint Task Force on Practice Parameters (JTFPP)

In a practice parameter concerning contact dermatitis, the Joint Task Force on Practice Parameters—composed of the American Academy of Allergy, Asthma & Immunology (AAAAI), the American College of Allergy, Asthma & Immunology (ACAAI), and the Joint Council of Allergy, Asthma & Immunology—proposed this series of summary statements:

"Summary Statement 1: Consider ACD [allergic contact dermatitis] in the differential diagnosis of patients with chronic eczematous or noneczematous dermatitis. [Strength of Recommendation: Strong; C Evidence]

Summary Statement 2: In patients suspected of ACD, patch testing is the gold standard to confirm the diagnosis. [Strength of Recommendation: Strong; C Evidence]

Summary Statement 3: In addition to personal products used by a patient suspected of ACD, review the home and workplace for other sources of contact allergens. [Strength of Recommendation: Moderate; D Evidence] Summary Statement

4: Evaluate patients for both irritant and allergic causes, especially in those presenting with hand dermatitis. [Strength of Recommendation: Strong; C Evidence]

Summary Statement 5: Allergic CD should be suspected and evaluated in the patient with both generalized and anatomically localized skin eruptions (such as the hands, face, eyelids) that come in contact with the substances in the environment. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 6: In a patient with a facial rash involving the periorbital areas (eg, eyelids), evaluate for ACD caused by components of cosmetics, such as fragrances, preservatives, and excipients, because these are common sensitizers of the facial skin. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 7: Evaluate patients presenting with lip dermatitis (cheilitis) and perioral dermatitis for both irritant and allergic causes of contact dermatitis. [Strength of Recommendation: Moderate; C Evidence]

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Summary Statement 8: Evaluate patients with chronic oral mucosal inflammatory conditions for disorders other than ACD. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 9: In patients presenting with dermatitis that involves the scalp and neck, consider patch testing for common causative sensitizers in cosmetics, hair products, and jewelry. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 10: Consider irritant and ACD in all patients presenting with acute or chronic hand eczema. All such patients suspected of CD should undergo patch testing. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 11: Evaluate patients with axillary dermatitis for ACD caused by local contact sensitivity to allergens in topically applied products found in deodorants and textiles. In some cases, axillary dermatitis could be a manifestation of systemic contact dermatitis (SCD) (ie, "the baboon syndrome"). [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 12: Evaluate patients presenting with anogenital dermatitis for possible ACD to antigens contained in topically applied products. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 13: Consider a diagnosis of SCD following systemic exposure (eg, ingestion, infusion, or transcutaneous exposure) to a known contact sensitizer in a patient who presents with generalized dermatitis, intertriginous and flexural exanthema (Baboon syndrome), and/or a flare at previous cutaneous sites of exposure [Strength of Recommendation: Moderate; C Evidence].

Summary Statement 14: Consider PT to rubber chemicals, adhesives, and leather components of footwear in patients presenting with unexplained chronic dermatitis involving the lower extremities, feet and/or soles. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 15: In addition to avoiding irritants in patients with atopic dermatitis (AD), evaluate for ACD, if suspected, as the 2 dermatologic conditions often coexist in the same patient. [Strength of Recommendation: Moderate; C Evidence]" (Fonacier et al., 2015).

Consensus based statements (CBSs) regarding the diagnosis and management of rhinitis from the JTFPP include the following:

Recommendation no.	CBS or GRADE recommendation	Strength of recommendation	Certainty of evidence
1	CBS: We recommend that the clinician complete a detailed history and a physical examination in a patient presenting with symptoms of rhinitis.	Strong	Low
2	CBS: We recommend that for patients presenting with rhinitis symptoms, a review of all current medications should be completed to assess whether drug-induced rhinitis may be present.	Strong	Ungraded
3	CBS: We recommend that aeroallergen skin prick testing or sIgE testing be completed to confirm the diagnosis of AR in a patient with a history consistent with AR.	Strong	High
4	CBS: We recommend that the clinician not perform food skin prick testing or sIgE for foods in their routine evaluation of a patient presenting with the signs and symptoms compatible with the diagnosis of AR.	Strong	Ungraded
5	CBS: We suggest that the use of a validated instrument (eg, scoring system, scale, or questionnaire) be considered to help determine the severity of rhinitis and to monitor the degree of disease control.	Conditional	Low

World Allergy Organization Position Paper

Allergen Testing, continued

In 2020, the World Allergy Organization published a position paper on IgE allergy diagnostics and other relevant allergy tests. Key statements from the paper can be found below:

"Clinical suspicion of allergic sensitization is confirmed by demonstrating the presence of allergen-specific IgE antibodies *in vivo* (skin tests) or *in vitro*.

Confirmation of allergen sensitization and the identification of causal allergens are essential for optimizing the management of allergic conditions.

Skin prick testing (SPT) is the most frequently used method for the detection of IgE antibodies, due to its rapidity, simplicity and low cost. Skin prick tests and other skin test results must be interpreted by a clinician with adequate knowledge of medical history, clinical findings, and relevant type I allergens (including environmental, food, animal, insect, fungal, and drug allergens). Skin tests should include the relevant allergens in the given geographical area and ideally carried out only using standardized allergenic extracts.

In vitro tests, including molecular based allergy diagnostics, using either in single-plex and in multi-plexed strategies and other more functional tests, such as Basophil Activation Tests allow to better define the IgE profile of the patient. This approach is in line with the Precision Medicine statements (I. J. Ansotegui et al., 2020)."

The paper also states that "Skin tests, especially SPT, represent the most reliable and cost-effective tool for the diagnosis and management of IgE-mediated diseases. They demonstrate a good correlation with outcomes of nasal, conjunctival, dermal, oral and bronchial challenges" (I. J. Ansotegui et al., 2020).

Clinical conditions where SPT is indicated include:

- "Asthma;
- Rhinitis/rhinosinusitis/rhino-conjunctivitis/conjunctivitis;
- Eczema/atopic dermatitis (in the setting of selectively high clinical suspicion for underlying presence of IgE hypersensitivity to specific allergens);
- Suspected food allergy (oral allergy syndrome, anaphylaxis/acute onset or exacerbation of urticaria or eczema that is temporally correlated with food ingestion);
- Suspected drug allergy;
- Hymenoptera venom allergy (systemic reactions immediately following insect sting);
- Suspected occupational disease or exposure to selected potential allergens;
- Chronic urticaria in rare selected cases which strongly suggest an allergen as potential trigger/ aggravating factor;
- Less common disorders, such as eosinophilic esophagitis, eosinophilic gastroenteritis or allergic bronchopulmonary aspergillosis, where IgE sensitization is one of the characteristics of its pathogenesis. However, there is controversy regarding the utility of SPT for these illnesses (I. J. Ansotegui et al., 2020)."

"SPT is not routinely indicated in the following instances in the absence of other existing features of allergic disease:

- Suspected food intolerance (e.g., irritable bowel syndrome, etc.);
- Chronic urticaria in the absence of allergic features in the history;

Allergen Testing, continued

- Desire to lose weight (according to nonconventional approaches, obesity may be due to food intolerance, but no supporting scientific data have been reported in the literature);
- Non-specific food-associated symptoms to food additives/preservatives/colorants;
- Evaluation of the effectiveness of allergen immunotherapy (but may be supportive in Hymenoptera venom immunotherapy);
- Non-specific respiratory symptoms to irritants (i.e., smoke, perfumes, detergents, chemicals and other strong odors);
- Screening for allergic sensitization patterns in the absence of clinical symptoms (i.e., family history of allergy);
- Non-specific cutaneous rashes in the absence of atopic features or other allergic symptoms; migraine, except for the indication of specific hypersensitivity to hormones. However, strong scientific data are still missing.
- Chronic fatigue syndrome (I. J. Ansotegui et al., 2020)."

In a 2020 publication on anaphylaxis guidance, the WAO confirms that "allergy testing should be based on patient history and local data regarding the common causes of anaphylaxis in the region. The most frequent elicitor groups worldwide are food, insect venom, and drugs" (Cardona et al., 2020).

World Allergy Organization (WAO), Allergic Rhinitis and its Impact on Asthma (ARIA), and the Global Allergy and Asthma European Network (GA2LEN)

The WAO, ARIA, and GA2LEN published a consensus document in 2020 focused on molecular-based allergy diagnoses. Precision allergy molecular diagnostic applications (PAMD@) "can increase the accuracy of an allergy diagnosis in certain circumstances. In allergic patients, a molecular approach is suitable for the following:

- assessing the risk of potential allergic reactions, which depend on the individual allergic (clinical) sensitization profile;
- evaluating whether unknown potential triggering factors are present (i.e., the presence of sIgE versus allergenic molecules correlated with high risk for allergic reactions)" (Ignacio J. Ansotegui et al., 2020).

National Institute of Allergy and Infectious Diseases (NIAID)

The NIAID convened an expert panel to review current information and to make recommendations related to the evaluation of food allergy (FA), including the use of specific IgE (sIgE) testing (Boyce et al., 2010). With regards to allergen-specific serum IgE determination, NIAID recommended that "sIgE tests for identifying foods that potentially provoke IgE-mediated food-induced allergic reactions, but alone these tests are not diagnostic of FA." It stated that "sIgE testing and skin prick testing both depend on the presence of allergen-specific antibodies. Because the former test measures sIgE in the serum and the latter reflects IgE bound to cutaneous mast cells, their results may not always correlate. Serum testing can be especially useful when SPTs cannot be done (for example, due to extensive dermatitis or dermatographism), or when antihistamines cannot be discontinued." The NIAID also recommended not using the combination of skin prick test (SPT), sIgE tests and atopy patch test (ATP) for the routine diagnosis of food allergy.

Additionally, the NIAID notes that "the routine use of measuring total serum IgE should not be used to make a diagnosis of FA."

Allergen Testing, continued

"Non-standardized tests" such as basophil histamine release/activation, lymphocyte stimulation, allergen-specific IgG, cytotoxicity assays, and mediator release assays should not be used in the routine evaluation of FA, according to the NIAID guidelines (Boyce et al., 2010).

In 2017, the NIAID published addendum guidelines for the prevention of peanut allergy in the United States. These guidelines note that the expert panel (EP) "recommends that evaluation with peanut-specific IgE (peanut sIgE) measurement, SPTs, or both be strongly considered before introduction of peanut to determine if peanut should be introduced and, if so, the preferred method of introduction. To minimize a delay in peanut introduction for children who may test negative, testing for peanut sIgE may be the preferred initial approach in certain health care settings, such as family medicine, pediatrics, or dermatology practices, in which skin prick testing is not routine" (Togias et al., 2017). Further, "The EP does not recommend food allergen panel testing or the addition of sIgE testing for foods other than peanut because of their poor positive predictive value, which could lead to misinterpretation, overdiagnosis of food allergy, and unnecessary dietary restrictions" (Togias et al., 2017). More, if an infant has severe eczema, an egg allergy, or both, the EP recommends to "Strongly consider evaluation by sIgE measurement and/or SPT and, if necessary, an OFC. Based on test results, introduce peanut-containing foods" (Togias et al., 2017).

American Academy of Pediatrics (AAP)

In 2012, AAP released a clinical report on allergy testing in childhood. It stated that "Both serum sIgE tests and SPT are sensitive and have similar diagnostic properties." The AAP summary included the following:

- "Treatment decisions for infants and children with allergy should be made on the basis of history and, when appropriate, identified through directed serum sIgE or SPT testing. Newer in vitro sIgE tests have supplanted radioallergosorbent tests."
- "Positive sIgE test results indicate sensitization but are not equivalent to clinical allergy. Large panels of indiscriminately performed screening tests may, therefore, provide misleading information."
- "Increasingly higher levels of sIgE (higher concentrations on serum tests or SPT wheal size) generally correlate with an increased risk of clinical allergy."
- "Use of a multiallergen serum test can be helpful for screening for atopic disease if there is a clinical suspicion. If positive, allergen-specific testing may be considered.
- "Tests for allergen-specific IgG antibodies are not helpful for diagnosing allergies (AAP, 2012)."

In 2019, the AAP published new guidelines on the prevention of childhood food allergies and other allergic conditions. This article states that "The new recommendations for the prevention of peanut allergy are based largely on the LEAP trial and are endorsed by the AAP." The AAP endorsed guidelines were published by Togias et al. (2017) and are noted above. They state that the highest-risk infants (those with severe eczema and/or egg allergies) should be introduced to peanuts by 4-6 months; further, allergy testing is strongly advised before peanut introduction. SPT and blood testing for peanut-specific IgE (sIgE) are allowable (Greer et al., 2019; S. Sicherer, 2017).

U.S. Food and Drug Administration (FDA) on Xolair

The availability of Xolair for treatment of allergic asthma also has implications for allergy testing. According to the package insert, "Xolair is indicated for adults and adolescents (12 years of age and above) with moderate to severe persistent asthma who have a positive skin test or in vitro reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids.

Determine doses (mg) and dosing frequency by serum total IgE level (IU/mL), measured before the start of treatment, and body weight (kg).” The prescribing information also notes that “Total IgE levels are elevated during treatment and remain elevated for up to one year after the discontinuation of treatment. Therefore, re-testing of IgE levels during Xolair treatment cannot be used as a guide for dose determination (FDA, 2016).”

International Consensus Statement on Allergy and Rhinology: Allergic Rhinitis

The authors reviewed the existing evidence behind various aspects of evaluation and diagnosis of the AR patient, and developed the following recommendations (Wise et al., 2018):

- “History taking is essential in the diagnosis of AR. Physical examination is recommended in the diagnosis of AR, and when combined with patient history, it increases diagnostic accuracy and excludes alternative causes. Making a presumptive diagnosis of AR on history (ideally combined with physical examination) is reasonable and would not delay treatment initiation. Confirmation with diagnostic testing is required for progression to AIT, or desirable with inadequate response to initial treatment.”
- “Skin-prick testing (SPT) is recommended for evaluation of allergen sensitivities in appropriately selected patients. Regular use of the same SPT device will allow clinicians to familiarize themselves with it and interpretation of results may therefore be more consistent. The use of standardized allergen extracts can further improve consistency of interpretation. Patients can benefit from identification of their specific sensitivities. SPT is a quick and relatively comfortable way to test several antigens with accuracy similar to other available methods of testing.”
- “Total IgE assessment is an option to assess atopic status. However, the evidence does not support a routine use.”
- “Serum sIgE testing may be used in the evaluation of AR. Using standardized allergens and rigorous proficiency testing on the part of laboratories may improve accuracy. Patients can benefit from identification of their specific sensitivities. Further, in some patients who cannot undergo skin testing, sIgE testing is a safe and effective alternative.”
- “The average pooled sensitivity of SPT is 85% which is often slightly higher than that of serum sIgE testing; however, this is not universally true depending on the allergen tested and the characteristics of the patient. Based on accuracy, convenience, cost, and promptness of results, SPT is often chosen as the first line diagnostic instrument to detect sensitivity to aeroallergens. Intradermal testing can be used as a second line test to exclude reactivity if the clinical suspicion is very high. In cases where dermatographism is present and/or patients are unable to wean off medications that affect skin testing, sIgE testing may be a better choice.”
- “BAT is an option for AR diagnosis when first-line tests are inconclusive or for measuring response to AIT. Basophil sensitivity may be a useful marker for following response to immunotherapy.”
- “Skin testing is not appropriate in all patients. Absolute or relative contraindications to SPT include uncontrolled or severe asthma, severe or unstable cardiovascular disease, concurrent beta-blocker therapy, and pregnancy. Certain medications and skin conditions may interfere with skin testing.”
- The list of medications that may interfere with skin testing are as follows: H1, H2, or topical antihistamines, anti-IgE (omalizumab), leukotriene receptor antagonists, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), benzodiazepines, topical (cutaneous) or systemic corticosteroids, and topical calcineurin inhibitors (ie. tacrolimus, picrolimus).

- The guideline states that because of the "lack of published studies on this topic, an Aggregate Grade of Evidence and evidence-based recommendation cannot be provided." However, they mention dermatitis and dermatographism as two skin conditions that may interfere with skin testing. (Wise et al., 2018)

The National Academies of Science, Engineering and Medicine (NASEM)

The National Academies of Science, Engineering and Medicine convened an expert committee to review the science and management practices of food allergy. Overall, they found that:

- "Currently, no simple diagnostic tests exist for food allergy."
- "Food allergy evaluation procedures include a medical history and physical examination, and also may include food-specific skin prick test, food-specific serum immunoglobulin E test, diagnostic food elimination diet, and oral food challenge (OFC). Selection of the specific tests needs to be individualized based on the medical history of each patient."
- "The BAT shows promising preliminary data, the potential utility is recognized and will require additional validation and standardization. "Guidelines suggest not using the BAT clinically on the grounds that it is nonstandardized, but recognize its use as a research tool (NASEM, 2016)."

In 2017, the National Academies of Science, Engineering and Medicine convened an expert committee to examine critical issues related to food allergy. Regarding diagnosis and prognosis, the committee notes that "physicians [should] use evidence-based, standardized procedures as the basis for food allergy diagnosis and avoid nonstandardized and unproven procedures….When food allergy is suspected, the patient should be evaluated by a physician who has the training and experience to select and interpret appropriate diagnostic tests" (S. H. Sicherer et al., 2017).

American Academy of Family Physicians (AAFP)

AAFP's recommendations for practice state: "Allergy and immunologic testing can help clarify the diagnosis and guide treatment. Immediate immunoglobulin E (IgE) and delayed T cell–mediated reactions are the main types of allergic responses. The allergens suspected in an immediate IgE-mediated response are identified through serum IgE-specific antibody or skin testing. For patients with an inhalant allergy, skin or IgE-specific antibody testing is preferred. In patients with food allergies, eliminating the suspected allergenic food from the diet is the initial treatment. If this is ineffective, IgE-specific antibody or skin testing can exclude allergens. An oral food challenge should be performed to confirm the diagnosis. Patients with an anaphylactic reaction to an insect sting should undergo IgE-specific antibody or skin testing. Skin testing for penicillin has a high negative predictive value and can help when penicillin administration is indicated and there are limited alternatives. Testing for other drug allergies has less well-determined sensitivity and specificity, but can guide the diagnosis. Patch testing can help identify the allergen responsible for contact dermatitis (K. L. Chang & J. C. Guarderas, 2018)."

European Academy of Allergy and Clinical Immunology (EAACI)

The EAACI published guidelines on "Biomarkers for monitoring the clinical efficacy of allergen Immunotherapy (AIT)." In it, they concluded that "to date, there are no validated and generally accepted candidate biomarkers that are predictive or indicative of the clinical response to AIT." However, they did note sIgE/tIgE ratio and IgE-FAB as candidate biomarkers for future research (Shamji et al., 2017).

The EAACI released a position statement on the BAT. In it, they concluded that "Basophil activation test has been established as a routine diagnostic test with standardized allergen preparations in a number of

service laboratories… An important next step is the standardization and automation of analysis of BAT. Once that is achieved, it will be possible to do large multicenter trials to characterize the diagnostic performance of BAT and broaden its use as a clinical tool (Hoffmann et al., 2015)."

National Institute for Health and Care Excellence (NICE)

NICE published a guideline on asthma, recommending against use of serum total or specific IgE for diagnosing asthma. Specific IgE or prick tests to aeroallergens should be used to identify triggers to asthma after a formal diagnosis has been made (NICE, 2021).

NICE also released a statement on multiplex allergen testing, particularly "ImmunoCAP ISAC" Although they acknowledge the test's promise, they state that there is "insufficient evidence to recommend the routine adoption of multiplex allergen testing with ImmunoCAP ISAC 112 to help diagnose allergy and predict the risk of an allergic reaction in people with allergy that is difficult to diagnose, when used with standard clinical assessment (NICE, 2020)."

Regarding the assessment and diagnosis of food allergy in under 19s, NICE published the below recommendations:

For food allergies classified as IgE-mediated:

"Based on the results of the allergy-focused clinical history, if IgE-mediated allergy is suspected, offer the child or young person a skin prick test and/or blood tests for specific IgE antibodies to the suspected foods and likely co-allergens."
"Tests should only be undertaken by healthcare professionals with the appropriate competencies to select, perform and interpret them."
"Skin prick tests should only be undertaken where there are facilities to deal with an anaphylactic reaction."
"Choose between a skin prick test and a specific IgE antibody blood test based on:

- the results of the allergy-focused clinical history **and**
- whether the test is suitable for, safe for and acceptable to the child or young person (or their parent or carer) **and**
- the available competencies of the healthcare professional to undertake the test and interpret the results."

"Do not carry out allergy testing without first taking an allergy-focused clinical history. Interpret the results of tests in the context of information from the allergy-focused clinical history."
"Do not use atopy patch testing or oral food challenges to diagnose IgE-mediated food allergy in primary care or community settings" (NICE, 2011).

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

Allergen Testing, continued

Code Number	Code Description
82784	Gammaglobulin (immunoglobulin); IgA, IgD, IgG, IgM, each
82785	Gammaglobulin; IgE
82787	Immunoglobulin subclasses (e.g., IgG 1, 2, 3, or 4) each
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method (ALCAT)
86001	Allergen specific IgG quantitative or semi-quantitative, each allergen
86003	Allergen specific IgE quantitative or semi-quantitative, each allergen
86005	Qualitative, multi-allergen screen (dipstick, paddle or disk)
86008	Allergen specific IgE; quantitative or semiquantitative, recombinant or purified component, each
88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker [anti-IgE receptor antibody testing] (BAT)
88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker
0165U	Peanut allergen-specific quantitative assessment of multiple epitopes using enzyme-linked immunosorbent assay (ELISA), blood, individual epitope results and probability of peanut allergy Proprietary test: VeriMAP™ Peanut Dx – Bead-based Epitope Assay Lab/Manufacturer: AllerGenis™ Clinical Laboratory
0178U	Peanut allergen-specific quantitative assessment of multiple epitopes using enzyme-linked immunosorbent assay (ELISA), blood, report of minimum eliciting exposure for a clinical reaction Proprietary test: VeriMAP™ Peanut Sensitivity - Bead Based Epitope Assay Lab/Manufacturer: AllerGenis™ Clinical 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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Biomarker Testing for Autoimmune Rheumatic Disease

Policy #: AHS – G2022	**Prior Policy Name & Number (as applicable):** ANA/ENA Testing (AHS-G2022)
Implementation Date: 9/15/21	**Date of Last Revision:** 4/28/22, 10/16/23 (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

Systemic autoimmune rheumatic diseases (SARDs) are a diverse group of conditions that primarily affect the joints, bones, muscle, and connective tissue (AAFP, 2019). SARDs are characterized by dysregulated immunity and inflammatory responses, resulting in damage and destruction to joints, connective tissues, skin, blood elements, and other target organs; however, considerable diversity in clinical presentation, disease course, and treatment response exists (Guthridge et al., 2022).

The diagnostic workup for SARDs may involve the antinuclear antibody (ANA) assay, which is used to detect autoantibodies (AAB) against intracellular antigens, originally known as antinuclear antibodies (Tan, 1989). Commonly used as part of the initial diagnostic workup to screen for evidence of systemic autoimmunity (Satoh et al., 2014), detection and identification of AABs are important in the diagnosis of SARDs, such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), mixed connective tissue disease (MCTD), systemic sclerosis (SSc), and idiopathic inflammatory myopathies (IIMs) (Tebo, 2017). Extractable nuclear antigens or ENAs (a historical term from when the antigens were extracted from the cell into saline solution prior to testing) include Sm, U1 ribonucleoprotein (RNP), Ro, and La antigens, and are also useful for evaluating individuals with suspected connective tissue disease (Bloch, 2022a).

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.

Biomarker Testing for Autoimmune Rheumatic Disease, continued

1. For individuals with a high clinical suspicion of autoimmune disease, testing for antinuclear antibodies (ANA) **MEETS COVERAGE CRITERIA:**

   a) Once per lifetime in individuals with stable symptoms.

   b) Repeat testing only if a significant change in symptoms occurs.

2. For individuals with an abnormal, raised ANA titer or with abnormal immunological findings in the serum and a clinical correlation with the appropriate autoimmune disorder, extractable nuclear antigens (ENA) panel testing of specific autoantibodies **MEETS COVERAGE CRITERIA.**

3. For individuals with an initial positive ANA test and a diagnosis of systemic autoimmune rheumatic disease, testing of dsDNA up to four (4) times per year **MEETS COVERAGE CRITERIA.**

4. For individuals with a negative or low positive ANA test, the following condition specific antibody testing **MEETS COVERAGE CRITERIA:**

   a) Testing for anti-Jo-1 in a unique clinical subset of myositis.

   b) Testing for anti-SSA in the setting of lupus or Sjögren's syndrome.

5. Monitoring of disease with ANA testing or ANA titers **DOES NOT MEET COVERAGE CRITERIA.**

6. For individuals with nonspecific symptoms, ANA and/or ENA testing **DOES NOT MEET COVERAGE CRITERIA.**

7. For all other situations not described above, testing of specific antibodies in the absence of a positive ANA test **DOES NOT MEET COVERAGE CRITERIA.**

8. For asymptomatic individuals, testing of ANA and/or ENA during a wellness visit or a general exam without abnormal findings **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.

9. For the management of rheumatoid arthritis (RA), serum biomarker panel testing (e.g., Vectra DA Score, PrismRA) **DOES NOT MEET COVERAGE CRITERIA.**

10. For the diagnosis of systemic lupus erythematosus (SLE), the use of cell-bound complement activation products (e.g., AVISE Lupus) **DOES NOT MEET COVERAGE CRITERIA.**

11. For the diagnosis, prognosis, or monitoring of SLE or connective tissue diseases, serum biomarker panel testing with proprietary algorithms and/or index scores (e.g., AVISE CTD, AVISE SLE Monitor, AVISE SLE Prognostic) **DOES NOT MEET COVERAGE CRITERIA.**

III. Scientific Background

Autoimmune diseases occur when an individual's immune system mistakenly attacks his or her own tissue. This can lead to a variety of conditions and diseases which vary in severity. Autoimmune diseases are estimated to affect 5% of the US population (Sirotti et al., 2017), are associated with increased morbidity and mortality, and are among the leading causes of death (under 65 years) and disability for women in the US (Simon et al., 2017).

Systemic lupus erythematosus (SLE) is one of more than 80 known autoimmune disorders, affecting approximately 23.2/100,000 people in the United States (Rees et al., 2017). The Lupus Foundation in America recently reported that lupus affects approximately 1.5 million people in the United States (LFA, 2019). SLE can present with a wide range of clinical manifestations, typically related to connective-tissue disorders, and often mimics other illnesses (Zucchi et al., 2019). This autoimmune disorder leads to inflammation and irreversible damage in one or more organs, including the joints, skin, nervous system, and kidneys (Durcan et al., 2019). The cause of SLE is not entirely understood, but it is predicted to manifest due to a combination of genetic and environmental factors, such as vitamin D deficiency, sunburn, and/or viral infections (Finzel et al., 2018). SLE affects women more than men and is a challenging disease to diagnose because of a broad assortment of signs, symptoms, and serological abnormalities (Durcan et al., 2019). SLE morbidity can be attributed to both tissue damage, toxic treatments, and complications associated with treatments, such as immunosuppression, long-term organ damage due to corticosteroid therapy, and accelerated coronary artery disease (Durcan et al., 2019; Fava & Petri, 2019). An early SLE diagnosis is particularly challenging as early-stage tests lack specificity; further, clinical signs and symptoms often only appear after organ damage has occurred, indicating later stages of the disease (Thong & Olsen, 2017). SLE diagnoses are made based on lab findings, clinical manifestations, serology, and histology of impacted organs (Thong & Olsen, 2017). However, current SLE screening tests are notoriously unreliable (Bhana, 2022).

Rheumatoid arthritis (RA) affects more than one million adults in the United States. RA is characterized by chronic inflammation of the synovial tissue of joints, cartilage, and bone (Cohen et al., 2021; Johnson et al., 2019; Luan et al., 2021; Pappas et al., 2021; Scherer, Haupl, & Burmester, 2020). Pathological abnormalities in patients with RA includes chronic synovitis, which results in joint devastation (Johnson et al., 2019; Luan et al., 2021; Scherer et al., 2020). Cellular and humoral response aberrations result in autoimmunity; antibodies and rheumatoid factors against post-translational modified proteins (including modifications such as citrullination). As such, synthetic cyclic citrullinated peptides (CCP) have been developed for diagnostic use (Scherer et al., 2020). To date, the etiology of RA has not been fully elucidated, though recent studies have suggested that genetic, epigenetic, and environmental factors contribute to RA presentation (Johnson et al., 2019; Scherer et al., 2020). Due to the complexity of RA pathogenesis, there is no model drug to cure RA.

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, 2022). 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 et al., 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).

Table 1. Autoantibody recognition in systemic autoimmune disease

Antigen location	Antibody	Antigen	Disease	PRR recognition
Nuclear	Anti-Ro (SS-a)	Ro-RNP complex	SLE, Sjögren's syndrome	TLR7
	Anti-La (SS-B)	La antigen	SLE, Sjögren's syndrome	TLR7
	Anti-Sm	Small nuclear RNP	SLE	TLR7
	Anti-dsDNA	dsDNA	SLE	TLR9
	Anti-histone	Histones	SLE (drug-induced)	TLR2 and TLR4
	Anti-Scl-70	Topoisomerase I	Systemic sclerosis	
	Anti-centromere	Centromere	Systemic sclerosis/CREST syndrome	
Cytoplasmic/ mitochondrial	ANCA	Myeloperoxidase (p-ANCA) and proteinase 3 (c-ANCA)	Vasculitis, Wegener's granulomatosis	
	ACA	Cardiolipin	Antiphospholipid syndrome, SLE	NLRP3
Modified proteins	ACPA	Citrullinated proteins	RA	TLR4
	Anti-Carp	Carbamylated proteins	RA	
Extracellular	RF	RF (IgG)	RA	
	Lupus anticoagulant	Phospholipids	Antiphospholipid syndrome	TLR4?
	α3 Chain of basement membrane collagen (type IV collagen)	α3 Chain of basement membrane collagen (type IV collagen)	Goodpasture's syndrome	

ACA, anti-cardiolipin antibody.

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, 2022). Autoantibodies to citrullinated protein epitopes, such anti-cyclic citrullinated peptide (anti-CCP2), has also been a focus of biomarker research in RA. Both RF and anti-CCP2 have similar sensitivities for the diagnosis of RA, but anti-CCP2 is positive in 20%-30% of RA patients who are negative for RF (Shapiro, 2021). RA is a heterogenous condition, and no single biomarker is a reliable predictor of RA disease activity (Mc Ardle et al., 2015).

Currently, conventional synthetic disease modifying anti-rheumatic drugs (cDMARDs) are the first line of RA therapy. Unfortunately, some RA patients do not respond to cDMARDs and clinical guidelines suggest use of alternative therapies such as TNFi. TNFi is the most common targeted treatment for RA patients who fail to respond to cDMARDs (Bergman et al., 2020; Johnson et al., 2019; Luan et al., 2021; Pappas et al., 2021). TNFi treatment, however, is not without limitations. Unfortunately, the majority of patients fail to respond to TNFi treatment (measured by American College of Rheumatology (ACR)50-indicates 50% disease improvement) and only 10-25% achieve remission (Cohen et al., 2021; Curtis et al., 2014; Johnson et al., 2019; Pappas et al., 2021). Currently, there is no way to predict whether RA patients will respond to TNFi therapy, and approximately three months is needed to determine whether a patient is responding (Johnson et al., 2019; Pappas et al., 2021). Accordingly, there has been a push to create a personalized medicine approach to identify non-responders to enhance clinical outcomes (Johnson et al., 2019; Pappas et al., 2021).

The systems by which the immune system maintains tolerance to an individual's own antigens can be overcome by release of intracellular antigens following excessive cell death, ineffective clearance of

apoptotic debris, inflammation-induced modification of self-antigens, or molecular mimicry, leading to the production of antibodies against self-antigens or autoantibodies (AAB) (Suurmond & Diamond, 2015). Autoantibodies mediate both systemic inflammation and tissue injury and may play a role in the pathogenesis of many autoimmune diseases (Suurmond & Diamond, 2015). Generally, AAB development precedes the clinical onset of autoimmune disease (Damoiseaux et al., 2015) and has predictive value (Satoh et al., 2014); thus, AABs serve as good serological markers to screen for evidence of autoimmunity (Aggarwal, 2014). Autoantibodies can target a variety of molecules (including nucleic acids, lipids, and proteins) from many cellular localizations—nucleus, cytoplasm, cell surface, extracellular organelles (Suurmond & Diamond, 2015), and different specific AABs are associated with particular diagnoses, symptoms, unique syndromes, subsets of disease, and clinical activity (Satoh et al., 2014). See Table 1 from Suurmond and Diamond (2015), below:

Table 1. Autoantibody recognition in systemic autoimmune disease

Antigen location	Antibody	Antigen	Disease	PRR recognition
Nuclear	Anti-Ro (SS-a)	Ro-RNP complex	SLE, Sjögren's syndrome	TLR7
	Anti-La (SS-B)	La antigen	SLE, Sjögren's syndrome	TLR7
	Anti-Sm	Small nuclear RNP	SLE	TLR7
	Anti-dsDNA	dsDNA	SLE	TLR9
	Anti-histone	Histones	SLE (drug-induced)	TLR2 and TLR4
	Anti–Scl-70	Topoisomerase I	Systemic sclerosis	
	Anti-centromere	Centromere	Systemic sclerosis/CREST syndrome	
Cytoplasmic/ mitochondrial	ANCA	Myeloperoxidase (p-ANCA) and proteinase 3 (c-ANCA)	Vasculitis, Wegener's granulomatosis	
	ACA	Cardiolipin	Antiphospholipid syndrome, SLE	NLRP3
Modified proteins	ACPA	Citrullinated proteins	RA	TLR4
	Anti-Carp	Carbamylated proteins	RA	
Extracellular	RF	RF (IgG)	RA	
	Lupus anticoagulant	Phospholipids	Antiphospholipid syndrome	TLR4?
	α3 Chain of basement membrane collagen (type IV collagen)	α3 Chain of basement membrane collagen (type IV collagen)	Goodpasture's syndrome	

ACA, anti-cardiolipin antibody.

However, serum AAB are present in 18.1% of the general population, and titers are higher in females and increase with age (Selmi et al., 2016). Additionally, only in a few cases does the antibody titer correlates with the severity of clinical manifestations or the response to treatment (Damoiseaux et al., 2015). The use of ANA detection as a diagnostic test originated with the observation of the lupus erythematosus (LE) cell (Hargraves et al., 1948). Since then, several tests have been developed to detect these antibodies.

The indirect immunofluorescence (IIF) test is the most widely used assay for the detection of AAB and remains the reference method of choice (ACR, 2015). Detection of ANAs by the IIF technique demonstrates binding to specific intracellular structures within the cells, resulting in staining patterns reported using the consensus nomenclature and representative patterns defined by The International Consensus on ANA staining Patterns (ICAP) initiative (Chan et al., 2016) and the degree of binding reflected by the fluorescence intensity or titer (Tebo, 2017). The test takes advantage of a HEp-2 cell line, which have large, easy to visualize, nuclei and contain nearly all of the clinically important autoantigens, making these cells ideal for the detection of the corresponding AABs (Bloch, 2022b). The ANA IIF assay using HEp-

Biomarker Testing for Autoimmune Rheumatic Disease, continued

2 slide has a high sensitivity for screening of SARDs and efforts to harmonize the nomenclatures for testing and reporting (Chan et al., 2015) have made this a powerful screening tool (Tebo, 2017). The frequency of ANA in SLE and SSc is 95–100%, 50–70% in SJS and 30–50% in rheumatoid arthritis (RA) (Satoh et al., 2014); however, their isolated finding in an otherwise healthy individual has a low positive predictive value which needs to be integrated with other laboratory parameters and patient risk factors (Selmi et al., 2016). Disadvantages of the indirect immunofluorescence test include its labor-intensiveness, significant training requirements for competence, and subjectivity in titer and pattern recognition; moreover, because the staining pattern usually does not identify the responsible autoantibody, additional testing may be required (Bloch, 2022b; Tebo, 2017). Automated image analysis provides a viable option for distinguishing between positive and negative results although the ability to assign specific patterns is insufficient to replace manual microscopic interpretation (Yoo et al., 2017).

The antinuclear antibody (ANA) test is commonly used in the evaluation of autoimmune disorders, as these antibodies are responsible for attacking healthy or normal cells. More than 95% of individuals with SLE will have a positive ANA test (Bhana, 2022). However, ANAs are present in “a significant proportion of normal individuals and lacks specificity or prognostic value” (Thong & Olsen, 2017). In particular, approximately only 11-13% of individuals with a positive ANA test will actually have SLE, and approximately 15% will be completely healthy (Bhana, 2022). Other SLE diagnostic methods include the monitoring of anti-double-stranded DNA (anti-dsDNA), C3 and C4 complement levels, CH50 complement levels, erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) levels, antiphospholipid antibodies, and urine protein-to-creatinine ratios (Wallace & Gladman, 2022).

If SLE is suspected based on the clinical picture following a positive ANA screen, the sera should be tested for antibodies to double-stranded DNA (dsDNA). Anti-dsDNA antibodies are present in two-thirds of patients with SLE, and they have a good association with disease activity and lupus nephritis. Serial monitoring of anti-dsDNA antibodies has modest correlation with disease activity (Aggarwal, 2014).

A positive ANA screen should also be followed by identification of sub-specificities by screening for antibodies to extractable nuclear antigens (ENAs). ENAs were identified by using saline extract of nuclei as the antigen. Antibodies to ENA can be determined using double immunodiffusion, immunoblotting, enzyme-linked immunosorbent assays (ELISA), or bead-based assay using recombinant or affinity-purified antigens. Different ENAs have an association with different connective tissue diseases (Aggarwal, 2014).

Reflex tests for positive ANA screens have been proposed to improve appropriateness in diagnosis of SARDs and avoid unnecessary second level testing. For specific autoantibodies responsible for certain fluorescent ANA patterns, such as homogeneous, speckled, fine grainy (Scl70-like), nucleolar, centromeric or speckled cytoplasmic, the identification of precise autoantibody markers is considered essential while for others it is not deemed to be necessary (Tonutti et al., 2016). See Table 1 from Tonutti et al, 2016, below.

Biomarker Testing for Autoimmune Rheumatic Disease, continued

Table 1

ANA-reflex test procedure with titres ≥1:160 and typical patterns

ANA-IIF pattern on HEp-2 cells	Reflex test(s)
Nuclear homogeneous ≥1:160	Antibodies to intracellular specific antigens (ENA) and to dsDNA/nucleosomes
Nuclear speckled ≥1:160	Anti-dsDNA and antibodies to intracellular specific antigens (ENA), possibly including anti-RNA polymerase III
Nuclear Scl70-like ≥1:160	Antibodies to intracellular specific antigens (ENA) (possibly including anti-PM/Scl)
Cytoplasmic speckled ≥1:160	Antibodies to intracellular specific antigens (ENA), including anti-tRNA synthetases and anti-P ribosomal
Pleomorphic PCNA-like (any titre)	Anti-PCNA
Centromere	No confirmation necessary if high titres. Execute specific test for anti-CENP B only in dubious cases (low titre or centromeric pattern not clearly recognizable)

ENA includes SS-A/Ro52 and Ro60, SS-B/La, Sm, RNP, Jo-1, and Scl70

Proprietary Testing

Proprietary tests exist for the assessment of SLE. For example, the "SLE-key" by ImmunArray is a molecular diagnostic test that is intended to help rule out an SLE diagnosis. This test determines the pattern of circulating antibodies and compares it to the proprietary pattern of antigens, "iCHIP". The pattern is compared to both SLE-affected and healthy control patterns, and an algorithm is used to assess the patient's likelihood of being affected with SLE. iCHIP was developed based on 250 affected and 250 healthy patients, and out of a 163 patient sample, the key was validated to "rule out" SLE at 94% sensitivity, 75% specificity, and 93% negative predictive value (ImmunArray, 2016, 2017). Another set of proprietary tests offered are from Exagen, under the "AVISE" line. Their line of tests utilizes a two-tiered testing method and a novel algorithm that measures 10 SLE relevant markers to deliver an index calculation value suggestive of the presence or absence of SLE. This includes tests for prognosis (10 biomarkers including various autoantibodies such as anti-C1q and antiribosomal P), diagnosis (10 biomarkers, includes ENA panel), and monitoring (6 biomarkers, includes anti-dsDNA and anti-C1q). AVISE CTD (standing for connective tissue disease) is intended to assist with the differential diagnosis of several autoimmune diseases and includes several ANA biomarkers, as well as an ENA panel. Other tests offered, such as AVISE Anti-CarP (evaluates autoantibodies to carbamylated proteins for rheumatoid patients) still include ANA components (AVISE, 2022).

AVISE Lupus by Exagen is a laboratory developed test (LDT) designed to assist in SLE diagnoses. This LDT utilizes a two-tiered testing method and a novel algorithm that measures 10 SLE relevant markers to deliver an index calculation value suggestive of the presence or absence of SLE. The AVISE Lupus test also uses cell-bound complement activation products (CB-CAPs) to measure complement system activation (Exagen, 2022). The 10 SLE relevant markers in this test include anti-dsDNA, anti-Smith (anti-Sm) antibodies, erythrocyte-bound C4d or B-lymphocyte-bound C4d (BC4d), ANA, CB-CAPs, and autoantibody specificity components (Exagen, 2022). As noted on their website, "The AVISE Lupus test is an ideal test for ANA positive patients with a clinical suspicion of lupus" (Exagen, 2022).

PrismRA is a molecular signature test that predicts TNFi non-response prior to treatment initiation. PrismRA utilizes a 23-feature blood-based molecular signature response classifier (MSRC) which integrates next generation RNA sequencing data and clinical features (clinical metrics, demographic variables, C reactive protein (CRP) and anti-CCP antibodies) to predict patients' response to TNFi treatment (Cohen et al., 2021). A high score is indicative of decreased likelihood of the RA patient to respond to TNFi therapies.

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 et al., 2013). While multi-biomarker panels are emerging as a potentially useful tool in the management of RA, there is not yet a consensus as to their clinical utility (Taylor & Maini, 2022).

Analytical Validity

A variety of manual or automated single or multiplex immunoassays have been introduced to make the process of detecting autoantibodies more efficient, including enzyme-linked immunoabsorbant assays (ELISA), fluorescent microsphere assays, and chemiluminescence immunoassays (CIA)—each with different performance characteristics (Tebo, 2017). In these assays, a panel of purified native or recombinant autoantigens is prepared, and each antigen is immobilized on a solid surface (microtiter plate, fluorescent microsphere, or membrane) and incubated with diluted human serum (Bloch, 2022b). The advantages of these alternative approaches to ANA IIF testing include their suitability for high-throughput testing, semi-quantification of test results, the lack of subjectivity, and the consolidation of ANA-related tests in a single platform as a positive test also provides identification of the responsible autoantibody (Bloch, 2022b; Tebo, 2017). It has been estimated that solid phase assays may decrease the labor cost of ANA testing by as much as 95 percent (Bloch, 2019). In a recent study which evaluated the performance of an automated CIA and fluorescence enzyme immunoassay (FEIA) and compared their performance to that of IIFA, both FEIA and CIA screen significantly outperformed IIF, with a higher specificity for FEIA and higher sensitivity for CIA (van der Pol, Bakker-Jonges, Kuijpers, & Schreurs, 2018). The use of solid phase assays as the initial test for the detection of ANA is concerning because the number of autoantigens that are included in solid phase assays is limited compared with the number that are present in the HEp-2 cell substrate, thus limiting sensitivity (Bloch, 2022b). Consequently, IIF remains the gold standard, and in cases of strong clinical suspicion of SARD and a negative screen from a solid phase assay, IIF should be performed (van der Pol et al., 2018).

Tipu et al. (2018) investigated the specificity and pattern for ANA in systemic rheumatic disease patients. 4347 samples were sent, and 397 were positive for ANA. Of these 397, 96 were positive on the anti-ENA screen and tested for anti-ENA reactivity. Anti-SSA antibodies were found in 59 of these samples. The most common ANA patterns were “coarse” and “fine-speckled” (43 and 22 of 81 respectively). However, no specific ANA pattern was associated with anti-ENA reactivity (Tipu & Bashir, 2018).

Kim et al. (2019) performed a meta-analysis comparing ANA measurement by automated indirect immunofluorescence (AIIF) and manual indirect immunofluorescence (MIIF). 22 studies including 6913 positive and 1818 negative samples of manual indirect immunofluorescence (MIIF) were included. Among this cohort, 524 samples with combined systemic rheumatic diseases (SRDs), 132 systemic lupus erythematosus (SLE) samples, and 104 systemic sclerosis (SSc) samples, and 520 controls were available. Positive concordance (PC) between AIIF and MIIF was 93.7%, although PC of total pattern and titer were lower. Clinical sensitivities of AIIF vs MIIF were 84.7% vs 78.2% for combined SRDs, 95.5% vs 93.9% for SLE, and 86.5% vs 83.7% for SSc. Clinical specificities of AIIF vs MIIF were 75.6% vs 79.6% for combined SRDs, 74.2% vs 83.3% for SLE, and 74.2% vs 83.3% for SSc. The authors concluded that the sensitivities did not differ between methods, but the specificities of SLE and SSc were statistically significant changes (Kim et al., 2019).

Dervieux et al. (2017) performed the analytical validation of Exagen's multianalyte panel test for SLE. This assay uses quantitative flow cytometry to assess the levels of the complement split product C4d bound to erythrocytes (EC4d) and B-lymphocytes (BC4d), in units of mean fluorescence intensity (MFI), and immunoassays to assay for antinuclear and anti-double stranded DNA antibodies (e.g. autoantibodies). The results were reported on a two-tiered index score as either positive or negative. The authors included specimens from both patients with SLE as well as individuals without SLE. Controls consisting of three-level C4 coated positive beads were run daily. The authors note that at ambient temperature both EC4d and BC4d are stable for 2 days and for 4 days if the samples are stored at 4˚C. "Median intra-day and inter-day CV [coefficient of variation] range from 2.9% to 7.8% (n=30) and 7.3% to 12.4% (n=66), respectively. The 2-tiered index score is reproducible over 4 consecutive days upon storage of blood at 4°C. A total of 2,888 three-level quality control data were collected from 6 flow cytometers with an overall failure rate below 3%. Median EC4d level is 6 net MFI (Interquartile [IQ] range 4-9 net MFI) and median BC4d is 18 net MFI (IQ range 13-27 net MFI) among 86,852 specimens submitted for testing. The incidence of 2-tiered positive test results is 13.4% (Dervieux et al., 2017)."

Putterman et al. (2014) compared the performance of C4d CB-CAPs on erythrocyte and B cells with antibodies to dsDNA, C3, and C4 in patients with SLE. A total of 794 individuals participated in this study, which included 205 healthy controls, 304 patients with SLE, and 285 patients with other rheumatic diseases. Both erythrocytes and B cells were measured with flow cytometry, and antibodies, including anti-dsDNA, were measured with solid-phase immunoassays. SLE activity was determined using the SLE Disease Activity Index SELENA Modification, and the two-tiered AVISE Lupus test was developed. Results showed that "The combination of EC4d and BC4d in multivariate testing methodology with anti-dsDNA and autoantibodies to cellular and citrullinated antigens yielded 80% sensitivity for SLE and specificity ranging from 70% (Sjogren's syndrome) to 92% (rheumatoid arthritis) (98%vs. normal)" (Putterman et al., 2014). Overall, the measurement of CB-CAPs was more sensitive for SLE diagnostic purposes than complement or anti-dsDNA measurements.

Ramsey-Goldman et al. (2020) evaluated the use of CB-CAPs, using flow cytometry, or a multianalyte assay panel (MAP) that includes CB-CAPs (e.g. AVISE Lupus) on patients with suspected SLE (n = 92) who fulfilled three classification criteria of the American College of Rheumatology (ACR). They also compared the data with individuals with established SLE (n = 53). At the initial visit, the individuals with suspected SLE had statistically higher positive CB-CAP (28%) or MAP results (40%) than individuals with established SLE. "In probable SLE, MAP scores of >0.8 at enrollment predicted fulfillment of a fourth ACR criterion within 18 months (hazard ratio 3.11, P<0.01)." The authors, who did acknowledge compensation from Exagen, conclude that "[a] MAP score above 0.8 predicts transition to classifiable SLE according to ACR criteria (Ramsey-Goldman et al., 2020)."

Clinical Utility and Validity

ANA, ENA, and SDLT

Oglesby et al. (2014) performed a cost-savings impact analysis on when the diagnosis of SLE is made and how it affects the clinical and economic outcomes. Using a claims database of claims made between January 2000 and June 2010, the authors separated individuals into two groups (n = 4166 per group) — early diagnosis (within 6 months of onset of symptoms) and late diagnosis (6 or more months after the onset of symptoms)—based upon an algorithm using a patient's ICD-9 diagnosis code(s) on the claim(s)

and when SLE medications were dispensed. Additional propensity scores were matched using data based on "age, gender, diagnosis year, region, health plan type, and comorbidities". Results show that the early diagnosis group had lower rates of mild, moderate, and severe flares as well as lower rates of hospitalization as compared to the late diagnosis group. Moreover, "[c]ompared with the late diagnosis patients, mean all-cause inpatient costs PPPM [per patient per month] were lower for the early diagnosis patients (US$406 vs. US$486; p = 0.016). Corresponding SLE-related hospitalization costs were also lower for early compared with late diagnosis patients (US$71 vs US$95; p = 0.013)." The values are adjusted to 2010 US dollars. The authors note that the other resource use and cost categories were consistent, concluding "[p]atients diagnosed with SLE sooner may experience lower flare rates, less healthcare utilization, and lower costs from a commercially insured population perspective (Oglesby et al., 2014)."

A study by Yeo et al. (2020) demonstrates that there is little benefit to repeat ANA testing if the initial test was negative by evaluating the cost of repeat ANA testing. From 2011 to 2018, 36,715 ANA tests were performed for 28,840 patients at a total cost of $675,029. Of these tests, 21.4% were repeats in which 54.9% of the patients initially tested negative. Of those who tested negative and repeated ANA testing, only 19% of the patients had a positive result when the test was repeated once in under two years, and this positive test did not lead to a change in diagnosis. Therefore, the authors conclude that "repeat ANA testing after a negative result has low utility and results in high cost" (Yeo et al., 2020).

Deng et al. (2016) investigated the clinical utility of ANA testing through different assays to see which one was most appropriate for evaluating patients with CTD. With 1000 samples collected, they compared an enzyme immunoassay (EIA), immunofluorescence assay (IFA), and multiplex immunoassay (MIA) in terms of specificity and sensitivity of testing. The researchers found that through using weights to define a patient sample that reflected the intended testing population and a normalized specificity of 90% to standardize the comparison between tests, the MIA, EIA, and IFA had sensitivities of 67%, 67%, and 56%, respectively. However, with a varying clinical cutoff, the IFA could obtain a sensitivity of 94% and a corresponding specificity of only 43%. This demonstrated that the sensitivity and specificity could easily vary with predetermined cutoffs; but, there were "no statistically significant differences in the clinical utility of the IFA, EIA, or MIA" (Deng et al., 2016).

Alsaed et al. (2021) compared the performance of ANA testing via ELISA vs IIF for CTDs. From a sample of 1457 patients and 12,439 tests ordered in 2016, they found that with "cut-off ratio ≥ 1.0 for ANA-ELISA and a dilutional titre ≥ 1:80 for ANA-IIF, the sensitivity of ANA-IIF and ANA-ELISA for all CTDs were 63.3% vs 74.8% respectively. For the SLE it was 64.3% vs 76.9%, Sjogren's Syndrome was 50% vs 76.9% respectively. The overall specificity of ANA-ELISA was 89.05%, which was slightly better than ANA-IIF 86.72%". This communicated the ELISA was slightly better than IIF in sensitivity and specificity, which could influence the convention of using IIF going forward if these findings are reflected in other cohort studies.

Biomarker analysis

Wallace et al. (2019) performed a randomized prospective trial to assess the clinical utility of the AVISE lupus MAP test (MAP/CB-CAP) as compared to standard diagnosis laboratory testing (SDLT). 145 patients with a history of positive antinuclear antibody status were randomly assigned to either an SDLT arm (n = 73) or the MAP/CB-CAP arm (n = 72) of the study. Treatment changes were recorded based on

either the SDLT or MAP/CB-CAP results. Even though the demographics between the two arms of the study were similar, the results were different. “Post-test likelihood of SLE resulting from randomisation in the MAP/CB-CAPs testing arm was significantly lower than that resulting from randomisation to SDLT arm on review of test results (−0.44±0.10 points vs −0.19±0.07 points) and at the 12-week follow-up visit (−0.61±0.10 points vs −0.31±0.10 points) (p<0.05). Among patients randomised to the MAP/CB-CAPs testing arm, two-tiered positive test results associated significantly with initiation of prednisone (p=0.034) (Wallace et al., 2019).” The authors conclude that testing such as the AVISE Lupus test has clinical utility and does affect treatment decisions.

A longitudinal, retrospective study by Mossell et al., (2016) of 46 patients who were anti-nuclear antibodies (ANA) positive but SLE-specific autoantibodies negative was conducted to evaluate the clinical utility of the AVISE Lupus test. 23 of the patients were in the “case” group (i.e. positive result based on the AVISE Lupus test), and 23 patients were in the “control” or negative results group. The charts of each individual were reviewed at two different times: T0 (or the initial time) and T1 (or approximately 1 year later). The case group was diagnosed with SLE at a higher rate than the control group (87% vs. 17%, respectively); moreover, the case group fulfilled 4 of the ACR classification criteria of SLE at a higher rate than the control group (43% vs 17%, respectively). The authors found that the sensitivity of the AVISE Lupus test (83%) is statistically significantly higher than the ACR score (42%, p = 0.006). Even at the initial baseline, individuals in the case group were prescribed anti-rheumatic medications more frequently (83% vs. 35%, p = 0.002) than the control group, indicating that a positive AVISE Lupus test may result in a more aggressive early treatment therapy (Mossell et al., 2016).

Liang et al. (2020) assayed the utility of the AVISE test in predicting lupus diagnosis and progression in 117 patients who previously did not have a diagnosis of SLE. The study assessed the patients at the time of the initial AVISE test (t = 0) and two years later (t = 2) using the SLE diagnosis criteria of the Systemic Lupus International Collaborating Clinics (SLICC) and ACR and the SLICC Damage Index (SDI) to measure SLE damage. After two years, patients who tested positive developed SLE at a significantly higher rate than those who tested negative using the AVISE test (65% vs 10.3%, p < 0.0001). AVISE-positive patients have more SLE damage after two years than AVISE-negative patients (1.9±1.3 vs 1.03±1.3, p=0.01). In particular, the authors note that the levels of BC4d “correlated with the number of SLICC criteria at t=0 (r=0.33, p< 0.0001) and t=2 (r=0.34, p<0.0001), as well as SDI at t=0 (r=0.25, p=0.003) and t=2 (r=0.26, p=0.002) (Liang et al., 2020).”

Alexander et al. (2021) further validated the clinical utility of the AVISE lupus test via a systematic review of medical records of ANA-positive patients with positive (>0.1) or negative (<-0.1) MAP scores. They found that the “odds of higher confidence in SLE diagnosis increased by 1.74-fold for every unit increase of the MAP score” with statistical significance, demonstrating that the test still further solidifies a diagnosis of SLE and can help inform “appropriate treatment decisions.”

A study by Clarke et al. (2020) demonstrates the cost-effective management of systemic lupus erythematosus (SLE) using a MAP rather than SDLTs. The higher specificity of MAP allows for an earlier SLE diagnosis, prompt initiation of the appropriate therapy, and fewer unnecessary and costly hospitalizations or investigations. Current SDLTS, such as ANA tests, have a high diagnostic sensitivity, but a high false-positive rate. MAP combines complement C4d activation products on erythrocytes and B cells with SDLTs, with antibodies to nuclear antigens, dsDNA IgG (with Crithidia confirmation), Smith, Sjogren’s syndrome type-B (SS-B/La), topoisomerase I (Scl-70), centromere protein B (CENP), histidyl t-

RNA synthetase (Jo-1), and cyclic citrullinated peptites (CCP) to improve SLE diagnosis. MAP "yields improved overall diagnostic performance with a sensitivity and specificity of 80% and 86%, respectively, compared with a sensitivity and specificity of 83% and 76%, respectively, for SDLTs. Despite the lower sensitivity, the superior specificity of MAP (86%) over SDLTs (76%) results in a higher positive predictive value associated with MAP (36.75%) compared with SDLTs (26.02%)"(Clarke et al., 2020). The improved specificity of MAP resulted in a cost savings of $1,991,152 to a US commercial plan over a 4-year time horizon, which translates to $0.04 in per member per month (PMPM) savings (Clarke et al., 2020).

Clinical validation of PrismRA was conducted in the Comparative Effectiveness Registry to Study Therapies for Arthritis and Inflammatory Conditions (CERTAIN) study (Bergman et al., 2020; Mellors et al., 2020). The CERTAIN trial was conducted by the Consortium of Rheumatology Researchers of North America which consisted of 43 sites and 117 rheumatologists (Mellors et al., 2020). This prospective study analyzed baseline RNA sequencing and clinical assessments to determine the effectiveness of PrismRA to predict TNFi non-response. Evaluation of the clinical response to TNFi was performed at six months and was determined by ACR50. The CERTAIN study built and validated the biomarker panel used for MSRC analyses. The study found that PrismRA demonstrated a positive predictive value of 89.7%, a specificity of 86.8%, and a sensitivity of 50% (Mellors et al., 2020; Pappas et al., 2021).

Inadequate TNFi response predictions were further validated on integrated blood samples from CERTAIN and NETWORK-004 studies. NETWORK-004 was a 24-week blinded prospective study conducted at 73 sites to evaluate the ability of MSRC to identify TNFi non-responders at three and six months by ACR50 (evaluations were also conducted using other scales such as Disease Activity Score (DAS28)-CRP, and Clinical Disease Activity Index). CERTAIN samples were used for transcript biomarker feature selection (n=100) and cross validation of MSRC (n=245). In the NETWORK-004 cohort, MSRC validation was performed in samples from naïve (n=146) and TNFi exposed (n=113) patients. ACR50 of patients stratified by MSRC at six months according to prediction of an inadequate response to TNFi therapy had an odds ratio of 4.1 (95% CI 2.0–8.3; p value=0.0001). Patients with a non-response MSCR were 26 times less likely to achieve remission evaluated three months after TNFi therapy (Cohen et al., 2021). Both studies found that PrismRA was able to accurately predict TNFi non-responders according to multiple clinically validated measurement scales (Cohen et al., 2021; Mellors et al., 2020).

Bergman et al. (2020) preformed modeling of the projected improvements from PrismRA and determined that ACR50 improved in the stratified cohort (40%) compared to the unstratified patient cohort (30%) and decreased costs of ineffective treatment by 19%. Further, PrismRA was shown to be a better predictor of inadequate response to TNFi treatment than clinical metrics alone (Bergman et al., 2020). Pappas et al. (2021) conducted a 32-question decision-impact survey involving 248 rheumatologists to determine whether predictive tests such as PrismRA appear to have clinical utility in RA patients' ability to respond to TNFi therapy. The study demonstrated that rheumatologists overwhelmingly supported the clinical need of predictive technologies to determine whether RA patients would respond to TNFi therapies and that payers should provide coverage of predictive technology (Pappas et al., 2021).

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:

Biomarker Testing for Autoimmune Rheumatic Disease, continued

- 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 et al. (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 et al. (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 and 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).

In a combined analysis of the OPERA, SWEFOT, and BRASS studies in which a newer version of the MBDA score was validated, Curtis analyzed the prognostic value of the adjusted MBDA score for radiographic progression in RA. The new MBDA score, used in these three studies, adjusts for age, sex, and adiposity. Curtis evaluated associations of radiographic progression (ΔTSS) per year with the adjusted MBDA score,

7. Renal Disorder
8. Neurologic Disorder
9. Hematologic Disorder
10. Immunologic Disorder
11. Positive Antinuclear Antibody

The ACR published a statement on the Methodology of Testing for Antinuclear Antibodies (ACR, 2015), which states:

1. The ACR supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing.
2. Hospital and commercial laboratories using alternative bead-based multiplex platforms or other solid phase assays for detecting ANAs must provide data to ordering healthcare providers on request that the alternative assay has the same or improved sensitivity compared to IF ANA.
3. In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti La/SS-B, etc., should be standardized according to national (e.g, CDC) and/or international (e.g., WHO, IUIS) standards.
4. Laboratories should specify the methods utilized for detecting ANAs when reporting their results.

The above positions were reaffirmed in 2019 (ACR, 2019).

The ACR also have developed a list of 5 tests, treatments or services that are commonly used in rheumatology practice, but their value should be questioned. The ANA testing was the first on the final top 5 items list with level of evidence Grade 1C. In their review, the Task Force considered recommendations currently published by CAP, ACR, ISLM. They have issued the following recommendation: "Do not test antinuclear antibody (ANA) subserologies without a positive ANA and clinical suspicion of immune-mediated disease (Yazdany et al., 2013)." For their list of 5 things to question for pediatric rheumatology, two points pertain to ANA testing (Rouster-Stevens et al., 2014). "Do not order autoantibody panels unless positive ANAs and evidence of rheumatic disease. There is no evidence that autoantibody testing (including ANA and autoantibody panels) enhances the diagnosis of children with musculoskeletal pain in the absence of evidence of rheumatic disease as determined by a careful history and physical examination." It was also stated in the latter recommendation, "Do not repeat a confirmed positive ANA in patients with established JIA or SLE (Rouster-Stevens et al., 2014)." These guidelines were reviewed and reaffirmed in 2021.

European League Against Rheumatism/American College of Rheumatology (EULAR/ACR)

The EULAR/ACR published a joint guideline to develop new classification criteria for systemic lupus erythematosus (SLE). In it, they stated that antinuclear antibodies (ANA) "at a titer of ≥1:80 on HEp-2 cells or an equivalent positive test" was to be an "entry criterion": if absent, the condition is not SLE; if present, apply additive criteria such as leukopenia or oral ulcers. Antiphospholipid antibodies, complement proteins, and SLE-specific antibodies (anti-dsDNA antibodies, Anti-Smith antibodies) are all included as additive criteria for SLE diagnosis (Aringer et al., 2019).

American Academy of Pediatrics (AAP)

The AAP released guidelines through ChoosingWisely. In it, they state "Do not order antinuclear antibody (ANA) and other autoantibody testing on a child unless there is strong suspicion or specific signs of autoimmune disease." A positive ANA may occur secondary to polyclonal activation of the immune system following an infection, or it may be positive without any identifiable reason/disease.

Systemic Lupus International Collaborating Clinics (SLICC)

The 2012 SLICC Classification Criteria for SLE splits the 17 criteria into two divisions—either clinical or immunologic. An individual scoring at least a 4, including at least one clinical criterion and one immunologic criterion, is classified as having SLE. The criteria are cumulative and do not need to be concurrently expressed or present (Petri et al., 2012). Mosca et al. (2019) also analyzed the accuracy and validity of the SLICC classification criteria, using a cohort of 616 patients, reporting an accuracy of 83.1%, sensitivity of 83.5%, and specificity of 82.4%. The criteria include the following (Petri et al., 2012):

A. Clinical Criteria

1. Acute cutaneous lupus, such as lupus malar rash or subacute cutaneous lupus
2. Chronic cutaneous lupus, such as classic discoid rash or discoid lupus/lichen planus overlap
3. Nonscarring alopecia
4. Oral or nasal ulcers
5. Joint disease
6. Serositis
7. Renal criteria, such as urine protein-to-creatinine ratio representing 500 mg protein/24 hours or red blood cell casts
8. Neurologic criteria, such seizures, psychosis, myelitis, and so on
9. Hemolytic anemia
10. Leukopenia or lymphopenia
11. Thrombocytopenia

B. Immunologic Criteria

1. ANA
2. Anti-dsDNA
3. Anti-Sm
4. Antiphospholipid antibodies
5. Low complement (Low C3, Low C4, or Low CH50)
6. Direct Coombs test in the absence of hemolytic anemia

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

A search for "antinuclear" on the FDA website on February 8, 2021, yielded 26 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.

VI. 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 Proprietary test: Vectra®DA Lab/Manufacturer: Crescendo Bioscience, Inc.
86038	Antinuclear antibodies (ANA)
86039	Antinuclear antibodies (ANA); titer
86225	Deoxyribonucleic acid (DNA) antibody; native or double stranded
86235	Extractable nuclear antigen, antibody to, any method (e.g., nRNP, SS-A, SS-B, Sm, RNP, Sc170, J01), each antibody
0039U	Deoxyribonucleic acid (DNA) antibody; double stranded, high avidity
0062U	Autoimmune (systemic lupus erythematosus), IgG and IgM analysis of 80 biomarkers, utilizing serum, algorithm reported with a risk score
0312U	Autoimmune diseases (eg, systemic lupus erythematosus [SLE]), analysis of 8 IgG autoantibodies and 2 cell-bound complement activation products using enzyme-linked immunosorbent immunoassay (ELISA), flow cytometry and indirect immunofluorescence, serum, or plasma and whole blood, individual components reported along with an algorithmic SLE-likelihood assessment Proprietary test: Avise® Lupus Lab/Manufacturer: Exagen 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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ANA/ENA Testing, continued

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VIII. Revision History

Revision Date	Summary of Changes
4/28/22	Added CPT code 0312U (Autoimmune diseases (eg, systemic lupus erythematosus [SLE]), analysis of 8 IgG autoantibodies and 2 cell-bound complement activation products using enzyme-linked immunosorbent immunoassay (ELISA), flow cytometry and indirect immunofluorescence, serum, or plasma and whole blood, individual components reported along with an algorithmic SLE-likelihood assessment Proprietary test: Avise® Lupus Lab/Manufacturer: Exagen Inc), which is a not-covered code with Select Health.
10/16/23	Changed title of policy to "Biomarker Testing for Autoimmune Rheumatic Disease."; added criterion #1a: "Once per lifetime in individuals with stable symptoms" and #1b: "Repeat testing only if a significant change in symptoms occurs."; added new coverage criteria #9: "For the management of rheumatoid arthritis (RA), serum biomarker panel testing (e.g., Vectra DA score, PrismRA) **DOES NOT MEET COVERAGE CRITERIA.**"; and added CPT code 81490.

Biochemical Markers of Alzheimer Disease and Dementia

Policy #: AHS – G2048	**Prior Policy Name & Number (as applicable):** AHS – G2048 – Biochemical Markers of Alzheimer's 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

Alzheimer disease (AD) is a neurodegenerative disease defined by a gradual decline in memory, cognitive functions, gross atrophy of the brain, and accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles (Karch, Cruchaga, & Goate, 2014).

II. Related Policies

Policy Number	Policy Title
AHS-M2038	Genetic Testing for Familial Alzheimer Disease

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. Measurement of cerebrospinal fluid biomarkers of Alzheimer disease, including but not limited to tau protein, amyloid beta peptides, α-synuclein, or neural thread proteins, **DOES NOT MEET COVERAGE CRITERIA**.
2. Measurement of plasma and/or serum biomarkers of Alzheimer disease, including but not limited to tau protein, amyloid beta peptides, neural thread proteins, ApoE, and ApoE4, **DOES NOT MEET COVERAGE CRITERIA.**
3. Measurement of urinary biomarkers of Alzheimer disease, including, but not limited to, neural thread proteins, amyloid beta peptides, and urinary extracellular vesicle analysis **DOES NOT MEET COVERAGE CRITERIA**.
4. The use of multianalyte assays, algorithmic analysis, and/or any other tests not mentioned above for the prognosis, diagnosis, and/or management of Alzheimer disease or dementia **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Alzheimer disease (AD) is a devastating neurodegenerative disease with a strong genetic component and is the predominant form of dementia (60-70%). In 2021, over 55 million people lived with dementia worldwide, and this number is estimated to increase to 139 million by 2050 (WHO, 2021). The average lifetime risk of developing AD is 10–12%; this risk at least doubles with the presence of a first-degree relative with the disorder (Goldman et al., 2011). The genetic predisposition of AD, even for late-onset AD patients, is estimated to be 60–80% (Gatz et al., 2006). According to the Centers for Disease Control and Prevention (CDC), the total adjusted death rates in the U.S. varied according to ethnicity with white, non-Hispanics having a rate of 70.8 per 100,000 individuals as compared to 65.0 and 46.0 per 100,000 for non-Hispanic black and Hispanic individuals (Kramarow & Tejada-Vera, 2019).

Most patients develop clinical symptoms at or after the age of 65 (spontaneous or late-onset AD), however 2–10% of patients have an earlier onset of disease (early-onset AD) (Shea et al., 2016). AD is characterized by severe neuronal loss, aggregation of extracellular amyloid β plaques, and intraneuronal tau protein tangles, resulting in progressive deterioration of memory and cognitive functions and ultimately requiring full-time medical care (Frigerio & Strooper, 2016). There is an enormous burden on public health due to the high costs associated with care and treatment. Aside from drugs that temporarily relieve symptoms, no treatment exists for AD (Van Cauwenberghe et al., 2016).

Many genetic studies have recently identified that late-onset Alzheimer disease is associated with the apolipoprotein E (*APOE*), apolipoprotein J (*APOJ*), and sortilin-related receptor (*SORL*) genes mainly expressed by various types of glial cells such as microglia, oligodendrocytes, and astrocytes; this has helped AD-related research stray from neurons and toward glial cells and neuroinflammation (Arranz & De Strooper, 2019).

The pathological processes of AD and other degenerative dementias are likely well underway before clinical symptoms manifest, therefore, biomarkers may have potential utility in the early diagnosis of dementia (Peterson, 2022). Mild cognitive impairment (MCI) is an intermediate state between normal cognition and dementia, recognizable as an early manifestation of dementia. MCI due to AD is the most common type of MCI (Bennett et al., 2002).

Studies have examined the use of cerebrospinal fluid (CSF) markers for predicting conversion from MCI to dementia. The most replicated CSF biomarkers include tau protein or phosphorylated tau protein and amyloid

beta 42 (Aß42) peptide, which may be represented by a low ratio of Aß42 to Aß40 levels, or a low ratio of Aß42 to tau levels. However, these tests vary in sensitivity (36 to 100 percent) and specificity (29 to 91 percent), and in the types of assays used. Recent research notes that the Aß42/40 ratio should be used over the measurement of Aß42 alone, as this ratio gives a more accurate diagnosis when analyzing CSF AD biomarkers (Hansson et al., 2019). Currently, these markers are of marginal clinical utility and do not have an established role in the evaluation of patients in the clinical setting (Peterson, 2022; Wolk & Dickerson, 2022).

Other biomarkers in CSF such as cargo proteins (e.g. chromogranin-B, α-synuclein), carnosinase I, chromogranin A, and NrCAM (neuronal cell adhesion molecule) have been proposed to provide clinical value for assessment of AD. Levels of each of the above CSF proteins are found to be statistically different among clinically defined patient groups with different degrees of cognitive impairment. However, the absence of a clinical treatment makes this relatively invasive test of questionable clinical utility (Schaffer et al., 2015; Wolk & Dickerson, 2022).

Plasma levels of the E4 variant of apolipoprotein E (ApoE4) may be a less invasive option for diagnosing patients. *ApoE* facilitates the delivery of cholesterol and promotes neuronal functionality and decreased apoE4 levels associated with neuronal degradation are suggestive of AD (Farrer et al., 1997). However, results are inconsistent across various studies. The correlation between altered levels of *ApoE* and ApoE4 with AD pathology is still not definitive, and standardization of methods is needed (Schaffer et al., 2015).

Studies have been conducted comparing the telomere length of peripheral blood leukocytes with those in the cerebellum (Patel et al., 2011). The shortening of telomere length is indicative of chronic stress on the human body, common in AD patients. However, cerebellar telomere length is not considered a diagnostic tool to evaluate the risk of inherited AD (Patel et al., 2011). Moreover, many other diseases also contain pathologies that induce stress on the body, so results may be confounded with other underlying health problems (Schaffer et al., 2015).

High concentrations of neuronal thread protein (NTP), specifically AD-associated NTP (AD7c-NTP), in urine is found to be representative of AD pathology (Patel et al., 2011). NTP is a brain protein that interacts with antibodies produced against pancreatic thread protein (PTP), a protein that contains structural components highly similar to the fibrils found in neuronal plaques in AD patients (Blennow et al., 2012; Patel et al., 2011). Moreover, AD7c-NTP is reflective of neuronal cell dysfunction. Unfortunately, NTP is more useful in determining the progression of the disease in patients who already have AD and not for early diagnosis (Lonneborg, 2008; Schaffer et al., 2015).

Studies have also identified a potential relationship between nanoscale extracellular vesicles (exosomes) and AD. Researchers note that exosomes may be an important factor in the progression of AD pathogenesis, but first need to identify the underlying AD-related mechanisms (Jiang et al., 2019).

Other media, such as saliva, have been proposed to provide diagnostic information for AD. A total of 6,230 metabolites from saliva were tested, and 3 were found to differentiate between MCI, AD, and cognitively normal patients (Huan et al., 2018).

None of these tests or biomarkers are valid as a stand-alone diagnostic test. The lack of standardized techniques makes diagnostic accuracy across all scenarios difficult to achieve. Current AD diagnostic standards using evaluation of clinical presentation have maintained a high level of accuracy, combined with the lack of a clinical treatment make all early AD diagnostic tests and biomarkers of limited clinical utility (François, Bull, Fenech, & Leifert, 2019; Schaffer et al., 2015). However, research criteria have incorporated both molecular and topographic biomarker data into the research definitions of both symptomatic and pre-symptomatic forms of

AD, anticipating that once biomarkers become more standardized they will be incorporated into clinical diagnostic algorithms for AD (Morris et al., 2014; Wolk & Dickerson, 2018).

Proprietary Testing

Proprietary tests exist for assessment of AD biomarkers. C2N Diagnostics offers PrecivityAD, a blood test that measures the ratio of Aß42 to Aß40 and ApoE detection. C2N Diagnostics received a "Breakthrough Device Designation" from the FDA in January 2019 for their test measuring the ratio of Aß42 to Aß40 (C2N, 2019).

Clinical Utility and Validity

Dage et al. (2016) studied the correlation of tau protein levels (in plasma) with neuronal damage. A total of 378 cognitively normal (CN) patients were examined, along with 161 patients with mild cognitive impairment (MCI). Baseline plasma tau protein levels were measured. The authors found that plasma tau levels were higher in MCI patients compared to CN patients (4.34 pg/mL for MCI compared to 41.4 pg/ML for CN, p = .078). The authors also performed a regression accounting for age, gender, education, and ApoE, which suggested that higher plasma tau levels were associated with worse memory loss and abnormal cortical thickness (Dage et al., 2016).

Lewczuk et al. (2017) compared the ratio of Aβ42/40 to just Aβ42 as measurements of clinical AD. A total of 200 patients (150 PET-negative, 50 PET-positive for amyloid) were examined and compared to the positron emission tomography (PET) results. The authors found that the ratio of Aβ42/40 agreed more strongly with the PET results (89.4% concordance compared to 74.9% concordance for Aβ42 only). A larger area under the curve was found for the Aβ42/40 measurement compared to just Aβ42 (0.936 compared to 0.814). The authors concluded that "the CSF Aβ42/40 ratio is superior to Aβ42 alone as a marker of amyloid-positivity by PET" (Lewczuk et al., 2017).

Talwar et al. (2016) performed a meta-analysis on CSF ApoE levels in AD patients. Twenty-four studies, including 1064 AD cases and 1338 healthy controls, were reviewed. The authors found that although the total sample did not indicate a significant association between AD and ApoE levels, a subgroup analysis controlling for sample size (n > 43) indicated significantly lower ApoE levels in AD patients compared to controls. The authors considered CSF ApoE levels to have "potential" as an indicator of AD association (Talwar et al., 2016).

Wang et al. (2018) evaluated the clinical value of α-synuclein in MCI and AD. The investigators added α-synuclein and phosphorylated α-synuclein to a biomarker panel containing Aβ42, tau, and phosphorylated tau and evaluated the new panel's performance. A total of 729 CSF samples were taken. The phosphorylated version of α-synuclein was found to weakly associate with diagnosis at baseline, but total α-synuclein was not. CSF α-synuclein was found to predict the Alzheimer's Disease Assessment Scale-Cognitive, memory, executive function, and progression from MCI to AD. Longitudinal biomarker changes were not found to differ between groups. Overall, α-synuclein was found to potentially better predict AD changes better than the classic biomarkers (H. Wang et al., 2018).

Zhang et al. (2014) performed a meta-analysis focusing on urinary Alzheimer-associated neuronal thread protein (AD7c-NTP)'s diagnostic ability for AD. Nine studies were reviewed for probable and possible AD, and the authors evaluated AD7c-NTP's sensitivity at 0.87, specificity at 0.89, positive likelihood ratio at 8.13, and negative likelihood ratio at 0.15 (Zhang et al., 2014).

Wang et al. (2019) explored the potential of urinary extracellular vesicle (EV) biomarkers in neurological disorders, including AD, Parkinson Disease (PD), and Huntington Disease (HD). A discovery cohort of 50 individuals was used to create the initial set of EV proteins and a set of 108 individuals was used to further develop the list of biomarkers. The authors identified "hundreds" of commonly expressed EV proteins with stable expression. SNAP23 and calbindin were most elevated in PD cases, with an 86% prediction of diagnostic success in the discovery cohort and 76% prediction of diagnostic success in the replication cohort. Moreover, "Broad Gene set analysis (GSEA) further reveals a prominent link to Alzheimer's disease with 10.4% of the genes known to be down-regulated in the brains from patients with Alzheimer's disease identified in urinary EVs" (S. Wang et al., 2019).

Liu et al. (2018) examined the urinary metabolic profile of β-amyloid 25-35 (Aβ 25-35)-injected rats. This was intended to establish AD in the rats, allowing the impairment of spatial learning and memory to be tested in the rats after 8 weeks. The authors identified the characteristic AD symptoms after 8 weeks (cognitive dysfunction, hippocampus damage, Aβ formation and tau phosphorylation) as well as 45 altered metabolites involving 8 metabolic pathways. The investigators concluded that "pathogenesis of AD was mainly due to gut microbiome dysbiosis, inhibition of energy metabolism, oxidative stress injury and loss of neuronal protective substances" (Liu et al., 2018).

Fossati et al. (2019) studied the correlation of plasma tau with cerebrospinal fluid (CSF) tau and phosphorylated tau (P-tau). A total of 97 subjects were included (68 healthy controls and 29 AD patients). Plasma tau was found to be higher in AD patients compared to healthy controls (area under curve: 0.79). However, CSF tau and plasma tau were "poorly" correlated. The addition of plasma tau to the receiver operating curve of CSF tau increased the area under curve to 0.82 from 0.80 and increased the curve of P-tau to 0.88 from 0.87. The authors concluded that "adding plasma tau to CSF tau or P-tau improves diagnostic accuracy, suggesting that plasma tau may represent a useful biomarker for AD" (Fossati et al., 2019).

Tatebe et al. (2017) developed an immunoassay to quantify plasma p-tau181. Three cohorts were used to validate the assay. In the first cohort (20 AD patients, 15 controls), the tau levels were found to be higher in the AD patients (0.171 ± 0.166 pg/ml in AD versus 0.0405 ± 0.0756 pg/ml in controls). In the second cohort (20 Down Syndrome patients, 22 controls), the tau levels were higher in the Down Syndrome patients (0.767 ± 1.26 pg/ml in DS versus 0.0415 ± 0.0710 pg/ml in controls). Finally, in the third cohort (8 AD patients, 3 other neurological diseases), the tau levels were found to correlate well with the CSF tau levels (r^2 = 0.4525). Overall, the authors suggested that "that the plasma p-tau181 is a promising blood biomarker for brain AD pathology" (Tatebe et al., 2017).

Shen et al. (2019) completed a meta-analysis review of 170 studies to research the role of inflammatory markers in AD and MCI. Increased periphery levels, compared to controls, were found with many types of biomarkers including high-sensitivity C reactive protein, p<0.05; interleukin-6, p<0.005; soluble tumour necrosis factor receptor 1, p<0.005; soluble tumour necrosis factor receptor 2, p<0.005; alpha1-antichymotrypsin, p<0.005; IL-1β, p<0.05; soluble CD40 ligand, p<0.05; CSF levels of IL-10, p<0.05; monocyte chemoattractant protein-1, p<0.005; transforming growth factor-beta 1, p<0.05; soluble triggering receptor expressed on myeloid cells2, p<0.001; YKL-40, p<0.001; α1-ACT, p<0.001; nerve growth factor, p<0.005; and visinin-like protein-1, p<0.005 (Shen et al., 2019). The authors conclude that all of the significant relationships found in this large meta-analysis help to support "the notion that AD and MCI are accompanied by inflammatory responses in both the periphery and CSF" (Shen et al., 2019).

Palmqvist et al. (2019) analyzed two different, cross-sectional, multicenter studies (n=1079). The CSF Aβ42/Aβ40 ratio was used to identify AD via Elecsys immunoassays from Roche Diagnostics; further, plasma neurofilament light chain (NFL), heavy chain (NFH), and *APOE* genotype were also analyzed in the first cohort of patients

(n=842). “In cohort 1, plasma Aβ42 and Aβ40 predicted Aβ status with an area under the receiver operating characteristic curve (AUC) of 0.80 (95% CI, 0.77-0.83). When adding *APOE*, the AUC increased significantly to 0.85 (95% CI, 0.82-0.88)” (Palmqvist et al., 2019). Cohort 2 had similar results with a slightly higher AUC (0.86; 95% CI, 0.81-0.91). The authors conclude by stating that “Plasma Aβ42 and Aβ40 measured using Elecsys immunoassays predict Aβ status in all stages of AD with similar accuracy in a validation cohort. Their accuracy can be further increased by analyzing *APOE* genotype” (Palmqvist et al., 2019).

Kim et al. (2020) studied the diagnostic utility of multiplexed sensing to detect multiple AD biomarkers (t-tau, p-tau181, Aβ42, and Aβ40) in human plasma using densely aligned carbon nanotubes (CNT). The CNT sensor assay exhibited superior sensitivity and precision, enabling the platform to accurately quantify AD biomarkers despite the hundreds of other agents in the blood plasma. The densely aligned CNT sensor array was $10–10^3$ times more sensitive than the commercially available sandwich-type or enzyme-linked immunosorbent assay. The authors conclude that "by measuring the levels of t-tau/Aβ42, p-tau181/Aβ42, and Aβ42/Aβ40 in clinical blood samples, the sensor array successfully discriminates the clinically diagnosed AD patients from healthy controls with an average sensitivity of 90.0%, a selectivity of 90.0%, and an average accuracy of 88.6%" (Kim et al., 2020).

Simrén et al. (2021) studied the diagnostic and prognostic potential of plasma biomarkers in Alzheimer disease. Various biomarkers, including phosphorylated-tau181 (P-tau181), neurofilament light, amyloid-β (Aβ42/40), total-tau and glial fibrillary acidic protein, were analyzed in 99 cognitively unimpaired (CU) patients, 107 mild cognitive impairment (MCI) patients, and 103 Alzheimer disease (AD) patients. According to the results, P-tau181 significantly outperformed all biomarkers in differentiating AD dementia from CU. Higher P-tau181 value was associated with increased cognitive decline and gray matter loss in temporal regions. The authors conclude that "these findings highlight the potential value of plasma P-tau181 as a non-invasive and cost-effective diagnostic and prognostic biomarker in AD" (Simrén et al., 2021).

Qu et al. (2021) performed a systematic review and meta-analysis of 150 studies aiming to evaluate the effect of AD biomarkers on blood. The authors performed a “random-effects meta-analysis based on the ratio of means method and multivariable-adjusted effect estimates.” The results demonstrated that T-tau, P-tau and Nfl increased, and that AβPPR decreased from controls to amnestic MCI (aMIC) to AD. Aβ42, Aβ42/40, and P-tau217 all had valid diagnostic accuracy. The authors conclude that the significant changes in core blood biomarkers support that “biomarkers were strongly valid in identifying AD” (Qu et al., 2021).

Chen et al. (2021) performed a meta-analysis of 17 studies aimed at calculating the diagnostic accuracy of blood-based biomarkers. The authors compared the diagnostic odds ratio (DOR) of biomarkers between controls, AD, and aMCI conditions. When comparing AD and control groups, the plasma Aβ42 DOR was 32.2 (sensitivity = 88 %, specificity = 81 %), the plasma Aβ oligomer DOR was 29.1 (sensitivity = 80 %, specificity = 88 %), and the plasma tau DOR was 52.1 (sensitivity = 90 %, specificity = 87 %). When comparing aMCI and controls, the plasma Aβ42 DOR was 60.4 (sensitivity = 86 %, specificity = 90 %), and the plasma tau DOR was 49.1 (sensitivity = 79 %, specificity = 94 %). The authors conclude that blood-based biomarkers are “minimally invasive and cost-effective tools for detecting AD; however, the evidence for detecting aMCI was still limited” (Chen et al., 2021).

Yoong et al. (2021) performed a systematic review and meta-analysis of 13 studies aiming to address the prognostic utility of a new CSF biomarker: Neurogranin (Ng). Core CSF biomarkers such as Aβ42, T-tau, and P-tau can support AD diagnosis, but cannot predict AD progression. Ng has been shown to predict cognitive decline. The authors found evidence that CSF Ng can predict Mini-Mental State Examination (MMSE) decline in $Aβ^+$ MCI patients and the decline of memory and executive function in MCI. Additionally, CSF Ng/Aβ42 was also found likely

to predict cognitive decline. The authors conclude that CSF Ng may be an applicable AD biomarker, but more studies are required to validate 548309its use (Yoong et al., 2021).

V. Guidelines and Recommendations

National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA)

In 1984, the NINCDS and ADRDA developed clinical criteria for the diagnosis of AD. While evidence to date has used NINCDS/ADRDA's AD classification, in 2011, the National Institute on Aging and the Alzheimer's Association workgroup revised diagnostic criteria for diagnosis of dementia due to Alzheimer's disease (McKhann et al., 2011).

The biomarkers reviewed in this policy are included in a category among revisions to AD diagnostic criteria- "probable AD dementia with evidence of the AD pathophysiological process". However, the diagnostic criteria workgroup publication noted "we do not advocate the use of AD biomarker tests for routine diagnostic purposes at the present time. There are several reasons for this limitation: 1) the core clinical criteria provide very good diagnostic accuracy and utility in most patients; 2) more research needs to be done to ensure that criteria that include the use of biomarkers have been appropriately designed, 3) there is limited standardization of biomarkers from one locale to another, and 4) access to biomarkers is limited to varying degrees in community settings. Presently, the use of biomarkers to enhance certainty of AD pathophysiological process may be useful in three circumstances: investigational studies, clinical trials, and as optional clinical tools for use where available and when deemed appropriate by the clinician" (McKhann et al., 2011).

Alzheimer's Association

The Alzheimer's Association has initiated a quality control program for CSF markers, noting that "Measurements of CSF AD biomarkers show large between laboratory variability, likely caused by factors related to analytical procedures and the analytical kits. Standardization of laboratory procedures and efforts by kit vendors to increase kit performance might lower variability, and will likely increase the usefulness of CSF AD biomarkers" (Mattsson et al., 2011).

In 2013, the Alzheimer's Association published recommendations for operationalizing the detection of cognitive impairment in the primary care setting (Cordell et al., 2013). It stated that "the use of biomarkers (e.g., CSF tau and beta amyloid proteins, amyloid tracer positron emission tomography scans) was not considered as these measures are not currently approved or widely available for clinical use."

In 2018, a multidisciplinary group of the Alzheimer's Association published criteria for lumbar puncture and CSF testing in the diagnosis of AD The committee recommends CSF biomarker testing for six clinical indications deemed appropriate, as listed in the table on the next page.

Table 1: Clinical indications for appropriate use of LP and cerebrospinal fluid testing in the diagnosis of AD (Shaw et al., 2018)

No.	Indication	Ratings
1	Cognitively unimpaired and within normal range functioning for age as established by objective testing; no conditions suggesting high risk and no SCD [subjective cognitive decline] or expressed concern about developing AD	Inappropriate

Biochemical Markers of Alzheimer Disease and Dementia, continued

2	Cognitively unimpaired patient based on objective testing, but considered by patient, family informant, and/or clinician to be at risk for AD based on family history	Inappropriate
3	Patients with SCD (cognitively unimpaired based on objective testing) who are considered to be at increased risk for AD	**Appropriate**
4	Patients with SCD (cognitively unimpaired based on objective testing) who are not considered to be at increased risk for AD	Inappropriate
5	MCI that is persistent, progressing, and unexplained	**Appropriate**
6	Patients with symptoms that suggest possible AD	**Appropriate**
7	MCI or dementia with an onset at an early age (<65)	**Appropriate**
8	Meeting core clinical criteria for probable AD with typical age of onset	**Appropriate**
9	Symptoms of REM sleep behavior disorder	Inappropriate
10	Patients whose dominant symptom is a change in behavior (e.g., Capgras Syndrome, paranoid delusions, unexplained delirium, combative symptoms, and depression) and where AD diagnosis is being considered	**Appropriate**
11	Use to determine disease severity in patients having already received a diagnosis of AD	Inappropriate
12	Individuals who are apolipoprotein E (APOE) ε4 carriers with no cognitive impairment	Inappropriate
13	Use of LP in lieu of genotyping for suspected ADAD mutation carriers	Inappropriate
14	ADAD mutation carriers, with or without symptoms	Inappropriate

Abbreviations: AD, Alzheimer's disease; LP, lumbar puncture; REM, rapid eye movement; SCD, subjective cognitive decline; ADAD, autosomal dominant Alzheimer's disease; MCI, mild cognitive impairment.

The workgroup has also identified several gray areas where more research is needed. The authors note that "One question that will need further data is whether measuring a ratio of CSF Aβ42/40 yields better diagnostic performance than measuring Aβ42 alone. Another question is how to characterize neurodegeneration using CSF biomarkers, and whether neurodegeneration in the absence of positive amyloid biomarkers predicts progression in persons with MCI" (Shaw et al., 2018). Further, the authors also state that "much more work is needed to document the potential impact of CSF AD biomarker testing on clinical outcomes in patients across the spectrum of AD" (Shaw et al., 2018).

Expert Working Group for the EU Joint Program—Neurodegenerative Disease Research (JPND) BIOMARKAPD Program

An expert working group, comprised of 28 international members, was convened to develop recommendations for CSF AD biomarkers in the diagnostic evaluation of dementia. "The working group recommended using the CSF biomarkers in MCI as an add-on to clinical evaluation alone for predicting functional decline or progression to AD dementia and, based on the available evidence, the recommendation was strong. However, in comparison with the outcome of using hippocampal atrophy as a biomarker, the working group issued a weak recommendation to incorporate CSF biomarkers in the diagnostic workup compared with hippocampal atrophy.

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Because of insufficient evidence, the working group could not recommend CSF biomarkers as an alternative to FDG-PET or amyloid-PET in predicting future decline or conversion. The working group recommended using CSF biomarkers to inform future disease management, but the strength of this recommendation was weak because of the small amount of evidence (Simonsen et al., 2017)."

Six clinical questions were asked by Simonsen et al. (2017):

1. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers identify or exclude AD as the cause of MCI?"
   a. Final recommendation: N/A
2. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict conversion to AD dementia within 3 years?"
   a. Final recommendation: Yes, strong
3. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict functional or cognitive decline?"
   a. Final recommendation: Yes, strong
4. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers change disease management?"
   a. Yes, weak
5. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers improve patient well-being?"
   a. Yes, weak
6. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers reduce health care costs?"
   a. No, weak

Additional recommendations were made by Herukka et al. (2017) for CSF AD biomarkers in the diagnostic evaluation of mild cognitive impairment. The same six clinical questions were asked as above by Herukka et al. (2017):

1. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers identify or exclude AD as the cause of MCI?"
   a. Final recommendation: N/A
2. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict conversion to AD dementia within 3 years?"
   a. Final recommendation: Yes, strong
3. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict functional or cognitive decline?"
   a. Final recommendation: Yes, strong
4. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers change disease management?"
   a. Yes, weak
5. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers improve patient well-being?"
   a. Yes, weak
6. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers reduce health care costs?"
   a. No, weak

National Institute on Aging (NIA, NIH) and Alzheimer's Association

In 2011, the National Institute on Aging and Alzheimer's Association workgroups published guidelines for the diagnosis of AD. The authors also note that "Two fundamental issues about individuals with MCI may be answered by the use of biomarkers: (1) To establish support for the underlying etiology of the clinical syndrome in an individual with MCI, which will have major importance for choosing the correct therapy, when effective treatments are available. (2) To determine the likelihood of cognitive and functional progression for an individual MCI patient to a more severe stage of MCI or to dementia, and the likelihood that this progression will occur within a defined period (Albert et al., 2011)." The authors also note that "in these recommendations, CSF tau is considered to be a strong marker of the neuronal injury associated with AD. However, the two biomarkers in combination are extremely informative. Together with low CSF Aβ42, elevated CSF tau provides a high likelihood of progression to AD in patients with MCI; however, because many biochemical events may be associated with AD, the authors confirm that "Additional work in this area is needed to know how useful these markers will be" (Albert et al., 2011). In 2018, guidelines were published by the National Institute on Aging and Alzheimer's Association for the preclinical, mild cognitive impairment, and dementia stages of AD, and are intended for use in observational and interventional research, not routine clinical care. These guidelines state that "there is now a growing consensus that application of biomarkers should be harmonized conceptually across the disease continuum and that biomarkers of neurodegeneration are not equivalent to those reflecting amyloid and pathologic tau accumulation" (Jack et al., 2018). Further, regarding the guidelines noted above from 2011, the authors state that "Studies published since 2011 have reinforced the idea that certain imaging and CSF biomarkers are valid proxies for neuropathologic changes of AD…. additional research has highlighted the fact that measures of neurodegeneration or neuronal injury that are commonly used in AD research—magnetic resonance imaging (MRI), fluoro-deoxyglucose (FDG) PET, and CSF total tau (T-tau)—are not specific for AD but rather are nonspecific indicators of damage that may derive from a variety of etiologies, for example, cerebrovascular injury (Jack et al., 2018)." The authors also state that the "data firmly establish that more advanced disease defined by biomarkers predicts greater likelihood of and more rapid cognitive decline. Thus, a solid evidence base exists proving that combinations of biomarker abnormalities are useful for staging the Alzheimer's continuum" (Jack et al., 2018).

Global Biomarker Standardization Consortium (GBSC)

The GBSC of the Alzheimer's Association has noted that before biomarkers can be used in clinical practice, they "must be standardized and validated on a global scale" (GBSC, 2019).

American Academy of Neurology (AAN)

In 2018 guideline was issued as an update to the 2001 AAN guideline on mild cognitive impairment (MCI) and endorsed by the Alzheimer's Association. This guideline was reaffirmed in 2021 (AAN, 2021). The panel determined that the field of biomarkers is rapidly evolving. And, according to the panel, there are no biomarkers that could clearly predict progression in patients with MCI.

They have provided the following recommendations:

- Recommendation A7a

  "For patients and families asking about biomarkers in MCI, clinicians should counsel that there are no accepted biomarkers available at this time (Level B)."

- Recommendation A7b

"For interested patients, clinicians may discuss the option of biomarker research or refer patients or both, if feasible, to centers or organizations that can connect patients to this research (e.g., subspecialty centers, Trial Match, ClinicalTrials.gov) (Level C)."

In 2001, the Quality Standards Committee of the American Academy of Neurology issued a "Practice parameter: Diagnosis of dementia (an evidence-based review)." Relevant statements to the current policy include the following:

"...no laboratory tests have yet emerged that are appropriate or routine use in the clinical evaluation of patients with suspected AD. Several promising avenues genotyping, imaging and biomarkers are being pursued, but proof that a laboratory test has value is arduous. Ultimately, the putative diagnostic test must be administered to a representative sample of patients with dementia who eventually have pathologic confirmation of their diagnoses. A valuable test will be one that increases diagnostic accuracy over and above a competent clinical diagnosis."

"There are no CSF or other biomarkers recommended for routine use in determining the diagnosis of AD at this time" (Knopman et al., 2001)

Dementia with Lewy Bodies (DLB) Consortium

The DLB Consortium published a consensus report on the diagnosis and management of dementia with Lewy bodies, which are characteristic of Alzheimer's Disease and other neurological conditions. The Consortium states that "direct biomarker evidence of LB-related pathology is not yet available for clinical diagnosis" (McKeith et al., 2017).

Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry

The Federation published an update on cerebrospinal fluid (CSF) and blood biomarkers for neurodegenerative dementias. The Federation considers blood-based biomarkers to "offer an ideal complementary step to advanced CSF and neuroimaging biomarkers and can serve as the first-step in a multi-stage process", although these biomarkers still require validation and "a great deal of additional work" (Lewczuk et al., 2018).

International Working Group (IWG)

In 2014, Dubois et al. (2014) published a position paper which presents a new diagnostic algorithm for AD which states: "Aβ1–42 and tau (T-tau or P-tau) should be used in combination, and the CSF AD signature, which combines low Aβ1 and high T-tau or P-tau concentrations, significantly increases the accuracy of AD diagnosis even at a prodromal stage. This combination reaches a sensitivity of 90–95% and a specificity of about 90% in AD. CSF biomarkers cannot be used as standalone tests and should be interpreted in a larger clinical context with confounding factors taken into account. An important concern is the large variability in CSF measures between laboratories and across techniques, and the lack of agreement on cutoff thresholds. These variations have made direct comparison of study results difficult. Several programmes of standardisation, including the Alzheimer's Association Quality Control programme for CSF biomarkers, initiatives within the Joint Program for Neurodegenerative Diseases, and the Global Biomarker Standardisation Consortium, and by industry, will minimise between-laboratory variations in the future and allow identification of uniform cutoff levels."

The IWG describes specific biochemical evidence in their definitions of AD:

"In-vivo evidence of Alzheimer's pathology (one of the following)

- Decreased Aβ1–42 together with increased T-tau or P-tau in CSF

- Increased tracer retention on amyloid PET
- AD autosomal dominant mutation present (in PSEN1, PSEN2, or APP)"

United States Preventive Services Task Force (USPSTF)

In 2020, the USPSTF published a recommendations stating that "current evidence is insufficient to assess the balance of benefits and harms of screening for cognitive impairment in older adults" (Owens et al., 2020).

European Federation of Neurological Societies (EFNS)

The EFNS published updated guidelines in 2012 for the diagnosis and management of disorders associated with dementia. These guidelines state that "Routine CSF analysis may help to rule out or rule in certain infectious causes (Good Practice Point). CSF abeta 1-42/tau/p-tau assessment helps to differentiate AD (Level B). Assessment of CSF total tau and 14-3-3 protein is recommended in rapidly progressive dementia when sCJD is suspected (Good Practice Point)" (Sorbi et al., 2012).

Canadian Consensus Conference on the Diagnosis and Treatment of Dementia (CCCDTD)

In 2020, the CCCDTD released recommendations on the diagnosis and treatment of dementia. The guidelines state that "CSF analysis is not recommended routinely, but it can be considered in dementia patients with diagnostic uncertainty and onset at an early age (<65) to rule out Alzheimer's disease (AD) pathophysiology." The guidelines also state that "CSF analysis can also be considered in dementia patients with diagnostic uncertainty and predominance of language, visuospatial, dysexecutive, or behavioral features to rule out AD pathophysiology" (Ismail et al., 2020).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On February 15, 2018, the FDA released a statement concerning the advancement of the development of novel treatments for neurological conditions, including Alzheimer's disease. FDA Commissioner Scott Gottlieb, M.D., states, "Symptoms and progression of neurological diseases can also vary significantly across patients, and even within patients, and across organ systems. Some diseases, like Alzheimer's, may progress invisibly for years. Once clinical symptoms become apparent, significant function may already be lost. These issues can make drug development more challenging for companies and are deeply frustrating for patients and caregivers living with these serious and life-threatening conditions. The FDA recognizes the urgent need for new medical treatments for many serious conditions including neurological disorders such as muscular dystrophies, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), migraine and epilepsy. This requires us to become more nimble, collaborative and patient-focused. As part of our ongoing efforts to expand access to safe and effective treatment options across all disease areas and promote innovation, the FDA is modernizing multiple aspects of our drug regulatory programs – including how we communicate scientific and regulatory guidance for drug development (Gottlieb, 2018)." Concurrently, the FDA released a guidance for industry concerning AD for public comment for 90 days. Within the guidance, the FDA states, "FDA supports and endorses the use of diagnostic criteria that are based on a contemporary understanding of the pathophysiology and evaluation of AD… Important findings applicable to the categorization of AD along its continuum of progression include the presence of pathophysiological changes as measured by biomarkers, the presence or absence of detectable abnormalities on sensitive neuropsychological measures, and the presence or absence of functional impairment manifested as meaningful daily life impact the present with subjective complaints or reliable observer reports (FDA, 2018)." The final draft of the guidance should be released in the future after the public comment period has concluded.

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 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
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified

Biochemical Markers of Alzheimer Disease and Dementia, continued

0206U	Neurology (Alzheimer disease); cell aggregation using morphometric imaging and protein kinase C-epsilon (PKCe) concentration in response to amylospheroid treatment by ELISA, cultured skin fibroblasts, each reported as positive or negative for Alzheimer disease
0207U	Quantitative imaging of phosphorylated ERK1 and ERK2 in response to bradykinin treatment by in situ immunofluorescence, using cultured skin fibroblasts, reported as a probability index for Alzheimer disease (List separately in addition to code for primary procedure)
0289U	Neurology (Alzheimer disease), mRNA, gene expression profiling by RNA sequencing of 24 genes, whole blood, algorithm reported as predictive risk score Proprietary test: MindX Blood Test™ - Memory/Alzheimer's Lab/Manufacturer: MindX Sciences™ Laboratory/MindX Sciences™ Inc
0346U	Beta amyloid, Aβ40 and Aβ42 by liquid chromatography with tandem mass spectrometry (LC-MS/MS), ratio, plasma Protietary test: QUEST AD-Detect™, Beta-Amyloid 42/40 Ratio, Plasma Lab/Manufacturer: Quest Diagnostics

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Bone Turnover Markers Testing

Policy #: AHS - G2051	**Prior Policy Name & Number (as applicable):** AHS - G2051 – Bone Turnover Markers for Diagnosis and Management of Osteoporosis and Diseases Associated with High Bone Turnover
Implementation Date: 9/15/21	**Date of Last Revision:** 11/12/21, 2/13/23, 1/8/24 (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

Bone metabolism involves a continual, dynamic equilibrium between bone growth and resorption. Bone turnover markers (BTMs) are biochemical markers for assessment of bone formation or bone resorption. These markers may be useful in determining risk of fracture and bone loss (Rosen, 2019b).

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.

Bone turnover markers are listed in Note 1.

1) For individuals treated with bisphosphonates, measurement of bone turnover markers to assess an individual's compliance with bisphosphonate therapy or for fracture risk prediction **MEETS COVERAGE CRITERIA** at the following intervals:

a. To establish baseline levels before initiating bisphosphonate treatment

b. Every three months after initiation or change of therapy for the first year

c. Every two years when no medication changes have occurred

2) For individuals with osteoporosis, measurement of bone turnover markers to monitor teriparatide treatment **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) As a diagnostic test for osteoporosis, measurement of bone turnover markers **DOES NOT MEET COVERAGE CRITERIA**.

4) In the diagnosis and management of patients with other conditions associated with high rates of bone turnover, measurement of bone turnover markers **DOES NOT MEET COVERAGE CRITERIA**.

Note 1: Bone turnover markers include (Rosen, 2021a, 2021b; Talwar, 2020):

1. Bone formation markers
   a. Serum bone–specific alkaline phosphatase (BSAP/BALP)
   b. Serum osteocalcin (OC)
   c. Serum type 1 procollagen (C-terminal/N-terminal): C1NP or P1NP
2. Bone resorption markers
   d. Urinary hydroxyproline (HYP)
   e. Urinary total pyridinoline (PYD)
   f. Urinary free deoxypyridinoline (DPD)
   g. Urinary or serum collagen type 1 cross-linked N-telopeptide (NTX)
   h. Urinary or serum collagen type 1 cross-linked C-telopeptide (CTX)
   i. Bone sialoprotein (BSP)
   j. Serum Tartrate-resistant acid phosphatase 5b (TRACP5b)
   k. Cathepsin K

III. Scientific Background

The resorption and reformation of bone are normally tightly regulated and coupled so that bone mass does not change. Bone disease occurs when these processes are uncoupled (Rosen, 2021a, 2021b). Biomarkers involved in the processes of resorption or formation have been proposed as measures for prediction of future bone loss, fracture risk, and more. Resorption markers include pyridinium crosslinks (PYD, DPD), C- and N-telopeptides (CTX, ICTP, NTX), tartrate-resistant acid phosphatase (TRACP) 5b, and cathepsin K, while formation markers include procollagen type I propeptides (PICP, PINP), osteocalcin, and bone-specific alkaline phosphatase (BSAP, also known as BALP) (Rosen, 2021a, 2021bb).

Formation markers are characteristic of bone formation rate. PICP and PINP are carboxy- and amino-sides of the tropocollagen peptide, which is a precursor to type I collagen in bone. The serum concentration of these peptides reflects synthesis of new collagen. Osteocalcin is a component of osteoid, and BSAP is the alkaline phosphatase specific to osteoblasts. These biomarkers reflect the activity of osteoblasts. Of these markers, BSAP and PINP are considered the most clinically useful (Rosen, 2021a, 2021b).

Resorption markers are characteristic of bone resorption rate (breakdown of bone). Pyridinium crosslinks are components of bone collagen, C- and N- telopeptides are crosslinks between bone collagen molecules, TRACP is anchored to the osteoclasts that initiate bone resorption, and cathepsin K is involved in digestion of the organic matrix (Manolagas, 2022; Rosen, 2021a, 2021b). Of these markers, urinary NTX and serum CTX are considered the most clinically useful (Rosen, 2021a, 2021b).

The measurement and use of these biomarkers remain complicated. Biologic variability between and within patients is significant, as factors such as age, gender, body mass index, circadian rhythms, menstruation, smoking, time of food consumption, exercise, and more may influence the levels of BTMs (Rosen 2019a, 2019b). Moreover, assays used to measure these biomarkers vary considerably, as both urinary and serum samples have been used. Lack of standardization has limited the use of BTMs in the clinical setting (Rosen, 2021a, 2021b).

Vitamin D supplementation has been used in the past for musculoskeletal diseases in both a prevention and treatment capacity— but data on supplementation with vitamin D and any corresponding effects on bone resorption and formation has been inconclusive. One study that investigated the effects of vitamin D supplementation on bone turnover markers such as bone-specific alkaline phosphatase (bALP), osteocalcin (OC), C-terminal telopeptide (CTX), and procollagen type 1 N-terminal propeptide (P1NP) failed to show any significant impact of vitamin D on bone turnover markers (Schwetz et al., 2017), while another study noted “a small, but significant, decrease in the bone formation marker procollagen of type 1 amino-terminal propeptide (P1NP)—in the vitamin D group as compared to the placebo group” (Jorde et al., 2019).

Analytical Validity

Eastell et al. (2000) assessed the biological variability between serum and urinary N-telopeptides of type I collagen (NTX). 277 postmenopausal women were included, and urine and serum specimens were included to identify short-term variability. Long-term variability was determined by comparing NTX at baseline and at 2 months. The authors found the median short-term coefficient of variation (CV) was 13.1% for urinary NTX and 6.3% for serum NTX. Long-term CV% was found to be 15.6% for urinary NTX and 7.5% for serum NTX. The authors also observed that to be 90% confident that a decrease in NTX after antiresorptive therapy was not caused by variability alone, a 31% decrease in urinary NTX and a 14% decrease in serum NTX are needed (R. Eastell et al., 2000).

Seibel et al. (2001) described the results of an international proficiency testing program for biochemical bone markers among clinical laboratories. The authors sent out two urinary and two serum pools (both normal and increased concentrations of markers) to 79 laboratories. The CVs were as follows: “serum bone-specific alkaline phosphatase (n = 47 laboratories), 16–48%; serum osteocalcin (n = 31), 16–42%; urinary free deoxypyridinoline (n = 30), 6.4–12%; urinary total deoxypyridinoline and

pyridinoline (n = 29), 27–28%; urinary N-terminal cross-linked telopeptide of type I collagen (n = 10), 39%; serum C-terminal cross-linked telopeptide of type I collagen (ICTP; n = 8), 22–27%; urinary hydroxyproline (n = 13), 12%". The authors concluded that "even with identical assays and methods, results for most biochemical markers of bone turnover differ markedly among laboratories (Seibel et al., 2001)."

Schafer et al. (2010) assessed the laboratory reproducibility of urine N-telopeptide (NTX) and serum bone-specific alkaline phosphatase (BAP). The authors obtained serum and urine from five postmenopausal individuals and sent specimens to six labs over eight months. They found that "Longitudinal coefficients of variation (CVs) ranged from 5.4% to 37.6% for NTX and from 3.1% to 23.6% for BAP. Within-run CVs ranged from 1.5% to 17.2% for NTX." (Schafer et al., 2010).

Hlaing and Compston (2014) notes that "although automated platforms have substantially improved the analytical variability of bone turnover markers, reproducibility still varies substantially." The National Bone Health Alliance executed a project to standardize bone turnover marker collection procedures and reduce pre-analytical variability (Bauer et al., 2012). The results of that project and the IOF and IFCC Bone Marker Standards Working Group identification of PINP and CTX-I in blood to be the reference markers of bone turnover for the fracture risk prediction and monitoring of osteoporosis treatment (Vasikaran et al., 2011) have resulted in recommendations for standard sample handling and patient preparation (Szulc et al., 2017). Standardization and harmonization of clinical assays for bone turnover markers such as CTx and P1NP are ongoing (IFCC, 2018).

Clinical Utility and Validity

Johansson et al. (2014) performed a meta-analysis to "examine the performance characteristics of serum procollagen type I N propeptide (s-PINP) and serum C-terminal cross-linking telopeptide of type I collagen (s-CTX) in fracture risk prediction in untreated individuals in prospective cohort studies." Six studies were included. The authors identified a "significant" association between s-CTX and risk of fracture (gradient of risk [GR] = 1.18). The hazard ratio per standard deviation increase in s-PINP was found to be 1.23 and was unadjusted for bone mineral density. The association between s-CTX and fracture risk was found to be 1.23. The authors concluded that "there is a modest but significant association between BTMs and risk of future fractures" (Johansson et al., 2014).

Marques et al. (2016) "assessed whether circulating bone formation and resorption markers (BTM) were individual predictors for trabecular and cortical bone loss, periosteal expansion, and fracture risk in older adults aged 66 to 93". A total of 1069 participants were included. Bone formation was assessed by serum procollagen type I N propeptide (PINP) and osteocalcin, and bone resorption was assessed by C-terminal cross-linking telopeptide of type I collagen (CTX). Inter-assay coefficients of variation were <3% for all BTM. A total of 236 particpants sustained a fracture during the median follow-up of 11.7 years. The authors found that "increase in BTM levels was associated with faster cortical and trabecular bone loss at the femoral neck and proximal femur. Higher BTM levels were positively related with periosteal expansion rate at the femoral neck in men. Markers were not associated with fracture risk (Marques et al., 2016)."

Mederle et al. (2018) investigated the correlation between bone mass density (BMD) and "serum levels of BTMs (tartrate-resistant acid phosphatase-5b [TRAP-5b]), bone-specific alkaline phosphatase (BSAP),

in postmenopausal osteoporotic women as compared to healthy postmenopausal subjects." A total of 132 postmenopausal individuals with osteoporosis were included along with 81 healthy postmenopausal individuals. BSAP was found to have a sensitivity of 76.5% and specificity of 84.3% at a cutoff of 21.27 U/L, and TRAP-5b was found to have a sensitivity of 86.3% and specificity of 90.6% at a cutoff of 3.45 U/L. The authors concluded that "our study showed that BMD correlates negatively with BTMs and TRAP-5b presents a good specificity in identifying patients with postmenopausal osteoporosis (Mederle et al., 2018)."

Tian et al. (2019) performed a meta-analysis "to explore whether bone turnover biomarkers (BTMs), i.e., C-terminal telopeptide of type I collagen (CTX) and procollagen type I aminoterminal propeptide (PINP), are associated with fracture." Nine studies were included. PINP had a "significant" positive association with fracture (adjusted gradient risk [GR] = 1.28) after adjusting for confounders. CTX was also seen to associate with fracture (GR = 1.20). The authors concluded, "Our results indicate a statistically significant but modest association between BTMs (s-PINP or s-CTX) and future fracture risk after adjusting for BMD and clinical risk factors. The causal relationship between the two clinical conditions requires future validation with more standardized studies (Tian et al., 2019)."

Naylor et al. (2019) evaluated bone turnover markers (BTMs)' ability to monitor "offset of treatment with bisphosphonates (BP) in osteoporosis". This was done by comparing the changes in BTMs and total hip (TH) bone mineral density (BMD). CTX and PINP were the BTMs analyzed, and offset was defined by "an increase greater than the least significant change (LSC) and an increase above the reference mean value." Fifty women were included, and at 48 weeks after stopping BPs, "CTX was greater than the LSC for 66% of women and PINP 72%; CTX was above the reference mean for 64% of women and PINP 42%." The authors also found that the decrease in TH-BMD was greater for women with the largest increases in BTMs, compared to those with "continued suppression." The authors concluded that "The measurement of BTM after withdrawal of BPs is potentially useful to evaluate patients that are taking a pause from treatment. An increase in BTMs more than the LSC and/or reference mean reflects loss of treatment effect and identifies patients that are likely to have a decrease in BMD" (Naylor et al., 2019).

Massera et al. (2019) evaluated the associations of osteocalcin (OC) and C-telopeptide of type I collagen (CTX) with "long-term incidence of hip fracture in older women." A total of 1680 women from the population-based Cardiovascular Health Study were included, and over a median follow-up period of 12.3 years, 288 hip fractures occurred. The authors found that increasing levels of CTX up to the middle-upper range (hazard ratio = 1.52 per standard deviation increase), with increases past this range only incrementally increasing risk (hazard ratio = 0.8). The authors identified an "inverted U-shaped relationship with incident fracture after adjustment" when comparing quartiles to each other, and an association was only seen for the quartile 3 to quartile 1 comparison (hazard ratio = 1.63). In a subset with "available measures", both OC and CTX were "inversely associated with bone mineral density of the hip". The authors concluded that "CTX, but not OC, levels were associated with incident hip fracture in post-menopausal women, a relationship characterized by an inverted U-shape" (Massera et al., 2019).

Migliorini et al. (2021) performed a systematic review of clinical trials reporting data on biomarkers for postmenopausal osteoporosis. A total of 36,706 patients were included from randomized trials. Data on biomarkers and clinical outcomes such as BMD, t-score, rate of fractures and adverse events were analyzed. Authors found that greater values of bone alkaline phosphatase (bALP) were associated with

more vertebral and non-vertebral fractures. Greater values of urinary cross-linked N-telopeptides of type I collagen (NTx) at baseline were linked with an increase in adverse events at the last follow-up, and greater values of C-telopeptide of type I collagen at baseline were associated with more adverse events leading to discontinuation, gastrointestinal adverse events, musculoskeletal adverse events, and mortality. The authors concluded that the review "supports the adoption of BMTs during pharmacological therapy setting of patients suffering from osteoporosis" (Migliorini et al., 2021).

Wei et al. (2021) explored the relationship of procollagen type 1 N-terminal peptide (P1NP) and *β* cross-linked C-telopeptide of type 1 collagen (*β*-CTX) with bone mineral density (BMD) in postmenopausal women. "All postmenopausal women were selected from a community-based case-control study and P1NP and *β*-CTX were also collected and tested. The main correlation analysis was applied to explore the relationships of BMD, P1NP, and *β*-CTX." The results indicated that "of the 1055 post-menopausal women that were enrolled, the BMD at all sites kept a decrease continually with age ($P < 0.01$). In addition, the level of *β*-CTX increased significantly from 45 to 50 years old and remained at a high level in the later stage, while the level of P1NP changed little or even decreased with age. Logistic regression model showed that *β*-CTX has better ability to predict BMD than P1NP, as demonstrated by an area under the curve (AUC) of 0.63." In conclusion, P1NP and *β*-CTX are important markers to monitor bone metabolism (Wei et al., 2021).

IV. Guidelines and Recommendations

The Bone Health and Osteoporosis Foundation

In 2022, the National Osteoporosis Foundation updated their guideline for prevention and treatment of osteoporosis. Regarding biochemical markers of bone turnover, the guideline states:

Biochemical markers of bone turnover may:

- Predict rapidity of bone loss in untreated postmenopausal individuals.
- Predict extent of fracture risk reduction when repeated after 3-6 months of treatment with FDA-approved therapies.
- Predict magnitude of BMD increases with FDA-approved therapies.
- Characterize patient compliance and persistence with osteoporosis therapy using a serum CTX for an antiresorptive medication and P1NP for an anabolic therapy (least significant change [LSC] is approximately a 40% reduction in CTX.
- Potentially be used during a bisphosphonate holiday to suggest when medication should be restarted, although more data are needed to support this recommendation (LeBoff et al., 2022).

The North American Menopause Society (NAMS)

In 2021, the North American Menopause Society (NAMS) issued an updated position on the management of osteoporosis in postmenopausal individuals. NAMS stated:

"Bone turnover markers cannot diagnose osteoporosis and have varying ability to predict fracture risk in clinical trials. Bone turnover markers have been used primarily in clinical trials to demonstrate group responses to treatment" (NAMS, 2021).

"Although used by some osteoporosis specialists, the routine use of bone turnover markers in the evaluation of patients with osteoporosis is not recommended" (NAMS, 2021).

"Although changes in bone turnover markers are used by some specialists to assess adherence and effectiveness of therapy, routine use of bone markers is not recommended" (NAMS, 2021).

International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

In 2021, the IOF/IFCC published "*Practical Considerations for the Clinical Application of Bone Turnover Markers in Osteoporosis*"(Vasikaran et al., 2023). The authors concluded, "Serum PINP and β-CTX are useful for monitoring oral therapy in osteoporosis. Further studies for their application in managing offset of drug action after cessation of antiresorptive therapies with bisphosphonates and denosumab would be useful. Large-scale fracture risk prediction studies of PINP and β-CTX in various untreated population groups to assess how they interact with established risk factors used in risk calculators such as FRAX may help to include BTMs in such algorithms.

The B-ALP and TRACP-5b are least affected by renal failure and may be of potential use in assessment for osteoporosis in patients with CKD and monitoring such patients when treated. Studies of utility of TRACP-5b and B-ALP in fracture risk assessment as well as monitoring therapy and assessing offset of treatment effect in osteoporosis patients with CKD stages 3a-5D is warranted.

From an analytical point of view, standardization or harmonization of commercial assays for BTMs is important for collation of data from different studies and uniform application of decision limits and treatment targets in clinical guidelines. IOF-IFCC C-BM is pursuing these activities" (Vasikaran et al., 2023).

American Association of Clinical Endocrinologists and American College of Endocrinology

An update to the 2016 Guidelines for the Diagnosis and Treatment of Postmenopausal Osteoporosis was published in 2020. In it, the AACE/ACE state "Consider using bone turnover markers in the initial evaluation and follow-up of osteoporosis patients. Elevated levels can predict more rapid rates of bone loss and higher fracture risk", which is identical to the 2016 statement, but the 2020 edition is graded at an "A", up from "B" in 2016.

Similarly, the statement "Consider using bone turnover markers (BTMs) for assessment of patient compliance and efficacy of therapy. Significant reductions in BTMs are seen with antiresorptive therapy and have been associated with fracture reduction, and significant increases indicate good response to anabolic therapy" remains unchanged from the 2016 version.

Other relevant recommendations include:

- "Consider bone turnover markers at or below the median value for premenopausal women as a target for response to therapy for patients taking antiresorptive agents. Consider significant increases in bone formation markers as a pharmacologic response to anabolic therapy."
- "The ending of a bisphosphonate holiday should be based on individual patient circumstances such as… an increase in bone turnover markers."

Bone Turnover Markers Testing, continued

Overall, although the joint guidelines acknowledge that BTMs cannot diagnose osteoporosis, they note that "elevated levels can predict more rapid rates of bone loss" and "are associated with increased fracture risk independent of BMD [bone mineral density] in some studies". Further, automated immunoassays have improved BTMs' reproducibility, and "changes in markers have been associated with bone response to therapy and reduction of fracture risk". Despite the numerous analytical issues with BTM assessment (lack of standardization, high cost, et al.), the guidelines note that some experts routinely use BTMs in clinical practice. They also note that the preferred bone turnover markers are PINP for bone formation and CTX for bone resorption. And, in the situations when patients might experience renal insufficiency or when there are insurance issues, then bone-specific alkaline phosphatase may be used. The guidelines conclude that "BTMs are useful in certain situations, such as assessment of fracture risk and to provide early feedback to patients that their drug is or is not working, which leads to discussions pertaining to medication compliance, drug absorption, and/or therapeutic efficacy. BTMs do not need to be assessed in all osteoporosis patients" (Camacho et al., 2020).

Consensus Group Report, managed by Scientific Advisory Board of European Society on Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases

This working group was intended to "to provide guidance to clinicians on how to use BTMs in patient evaluation in postmenopausal osteoporosis, in fracture risk prediction and in the monitoring of treatment efficacy and adherence to osteoporosis medication". Their conclusions are listed below (Lorentzon et al., 2019):

- "The bone formation marker serum PINP [N-terminal collagen type I extension propeptide] and resorption marker serum βCTX-I [bone alkaline phosphatase for bone formation and C-terminal cross-linking telopeptide of type I collagen] are the preferred markers for evaluating bone turnover in the clinical setting."
- "Bone turnover markers cannot be used to diagnose osteoporosis but can be of value in patient evaluation and can improve the ability to detect some causes of secondary osteoporosis."
- "Serum βCTX-I and PINP correlate only moderately with bone loss in postmenopausal women and with osteoporosis medication-induced gains in BMD. Therefore, the use of bone turnover markers cannot be recommended to monitor osteoporosis treatment effect in individual patients."
- "Adding data on serum βCTX-I and PINP levels in postmenopausal women can only improve fracture risk prediction slightly in addition to clinical risk factors and BMD and therefore has limited value."
- "Bisphosphonates are the most commonly used osteoporosis medications, but adherence to oral bisphosphonates falls below 50% within the first year of treatment. Monitoring PINP and βCTX-I is effective in monitoring treatment adherence and can be defined as the sufficient suppression of these markers (by more than the LSC or to the lower half of the reference interval for young and healthy premenopausal women)".

The guideline remarks "It is possible that monitoring the bone marker response may aid in the use of bisphosphonate treatment frequency and dosing when denosumab treatment is stopped." (Lorentzon et al., 2019)

The guideline also notes that a "systematic review of the present evidence concluded that there is insufficient evidence to recommend the use of monitoring bone turnover markers for predicting the effect of teriparatide treatment effect" (Lorentzon et al., 2019).

U.S. Preventative Services Task Force (USPSTF)

The 2018 USPSTF recommendation on screening to prevent osteoporotic fractures address clinical risk assessment and bone density measurement but do not mention bone turnover markers. (Viswanathan et al., 2018)

Endocrine Society

The Endocrine Society released a guideline titled "*Pharmacological Management of Osteoporosis in Postmenopausal Women"*, which noted, "Monitoring bone turnover markers (serum C-terminal crosslinking telopeptide for antiresorptive therapy or procollagen type 1 N-terminal propeptide for bone anabolic therapy) is an alternative way of identifying poor response or nonadherence to therapy (Eastell et al., 2019)."

The Endocrine Society published an update to the above guideline in 2020, and the above statement concerning monitoring of bone turnover markers remained in the 2020 edition (Shoback et al., 2020).

The Endocrine Society also released guidelines regarding the management of Paget's Disease. They recommended "that in patients with increased bone turnover, biochemical follow-up should be used as a more objective indicator of relapse than symptoms" (Singer et al., 2014).

"For most patients, measurement of total alkaline phosphatase or other baseline disease activity markers at 6 to 12 weeks, when bone turnover will have shown a substantial decline, is an acceptable and cost-effective option" (Singer et al., 2014).

National Osteoporosis Guideline Group (NOGG)

The NOGG notes bone turnover markers (e.g., CTX, P1NP) as a possible measure to evaluate during investigation of osteoporosis/fragility fractures (NOGG, 2021).

Kidney Disease Improving Global Outcomes (KDIGO): Mineral and Bone Disorder

The KDIGO released guidelines pertaining to bone turnover related to CKD.

- "In patients with CKD [stages] G3a–G5D, we suggest that measurements of serum PTH or bone-specific alkaline phosphatase can be used to evaluate bone disease because markedly high or low values predict underlying bone turnover."
- "In patients with CKD [stages] G3a–G5D, we suggest not to routinely measure bone-derived turnover markers of collagen synthesis (such as procollagen type I C-terminal propeptide) and breakdown (such as type I collagen cross-linked telopeptide, cross-laps, pyridinoline, or deoxypyridinoline)" (KDIGO, 2017).

The **Renal Association** also published a "commentary" on the KDIGO guidelines in 2018. In it, they remarked that "Although iPTH, whole PTH, and bALP levels were associated with bone turnover, no biomarker singly or in combination was sufficiently robust to diagnose low, normal, and high bone turnover in an individual patient [on dialysis]." (Burton, Goldsmith, Ruddock, Shroff, & Wan, 2018)

Fourth International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism

This workshop published guidelines regarding management of asymptomatic primary hyperparathyroidism (PHPT). They note bone turnover markers as an optional measurement of asymptomatic PHPT, listing "bone-specific alkaline phosphatase activity, osteocalcin, P1NP [select one]; serum CTX, urinary NTX [select one]" (Bilezikian et al., 2014).

International Osteoporosis Foundation and European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis

The IOF/ESCEO issued joint guidelines stating the following (Kanis et al., 2018):

"Bone markers (serum procollagen type I N propeptide (s-PINP) and serum C-terminal cross-linking telopeptide of type I collagen (s-CTX) as markers of bone formation and bone resorption, respectively) have some prognostic significance for fracture in situations where bone mineral density (BMD) is unavailable."

The joint guidelines also note that if harmonization efforts for other bone turnover markers are successful, these markers may see use for fracture risk. Procollagen I N-terminal peptide (P1NP) and C-telopeptide breakdown products (especially serum CTX) are considered the most informative biochemical markers for monitoring of osteoporosis (Kanis et al., 2018).

International Osteoporosis Foundation (IOF), Asian Federation of Osteoporosis Societies (AFOS), and the International Society for Clinical Densitometry (ISCD)

The IOF Capture the Fracture program facilitates the establishment of Fracture Liaison Services (FLS) with a goal "properly identify and treat patients with fragility fractures, improve quality of post-fracture care, adherence, and prevention of secondary fractures worldwide, including the [Asia-Pacific] region." In 2021, the IOF, AFOS, and ISCD endorsed a consensus statement on the use of BTMs in the Asia-Pacific region. They made the following consensus statements (Wu et al., 2021):

- "Endorse the use of BTMs, especially CTX and P1NP, as short-term monitoring tools for osteoporosis treatment, consistent with recommendations of the AACE/ACE, IOF, IFCC, JOS, NOF, TOA, and associated organizations.
- BTMs can be used to differentiate patients with relatively higher or lower bone turnover rates and thereafter, helping clinicians to choose an appropriate anti-osteoporosis treatment regimen.
- BTMs can reflect the therapeutic responses to anti-osteoporosis therapies earlier than BMD and are therefore of help both in selecting osteoporosis treatment and in assessing its responses to therapies.
- Absolute values or the degree of change from baseline for BTMs can be used to monitor the efficacy of osteoporosis therapies clinically.
- CTX and/or P1NP can be used to evaluate patient adherence and drug responses to anti-resorptive agents, with measurements suggested at baseline, three months, six months, and 12 months after starting treatment.
- P1NP can be used to evaluate patient adherence and drug responses to anabolic agents, with measurements at baseline, one to three months, six months, and 12 months after starting anabolic treatment.

- Encourage reimbursement of BTMs by different health insurance programs in the Asia-Pacific to improve patient adherence and treatment outcomes.
- Recommend appropriate use of BTMs as a short-term monitoring tool for improving the use of therapeutic regimens in osteoporosis care programs, such as fracture liaison service (FLS)."

They conclude that "the use of BTMs can be incorporated in treatment algorithms of osteoporosis care programs to improve patient adherence and treatment outcomes" (Wu et al., 2021).

The International Federation of Clinical Chemistry and Laboratory Medicine

The most recent review of bone turnover markers for the journal of the International Federation of Clinical Chemistry and Laboratory Medicine (Bhattoa, 2018) found that "Although quite sensitive to a multitude of exogenous and endogenous pre-analytical factors, bone markers are best used in monitoring anti-osteoporosis therapy efficacy and compliance. Combination of BMD measurement by DEXA with biochemical markers of bone turnover levels, at least one bone resorption and one bone formation marker, may potentially improve early detection of individuals at increased risk for bone loss and eventually non-traumatic bone fracture. Furthermore, they have widespread clinical utility in osteoporosis, renal osteodystrophy, certain oncological conditions and rheumatic diseases."

International Society for Clinical Densitometry

The ISCD includes a comment on bone turnover markers in their guideline titled "Official Positions", stating that "Serial BMD [bone mineral density] testing in combination with clinical assessment of fracture risk, bone turnover markers and other factors…can be used to determine whether treatment should be initiated in untreated patients, according to locally applicable guidelines" (ISCD, 2019).

Royal Australian College of General Practitioners (RACGP)

In 2013, the RACGP released a series of "Tests and results" aimed at providing information about common tests that general practitioners order regularly. The series focused on areas such as indications, what to tell the patient, what the test can and cannot tell you, and interpretation of the results. As an assessment of fracture risk, they note that "Bone turnover markers increase in proportion to fracture risk, independent of bone mineral density (BMD). In general, turnover markers also tend to be higher in patients with low bone density. However, this correlation is not absolute in individuals and this application of the test is most useful in population studies. Very high marker levels (more than 1.5 times the upper reference limit) are not typical of postmenopausal osteoporosis and should prompt a search for another cause. For example, after a fracture, markers may remain increased for up to six months. Other causes could include high turnover states such as hyperparathyroidism or hyperthyroidism, Paget disease, malignancy including myeloma, or advanced renal failure" (Coates, 2013).

As a method of monitoring the efficacy of osteoporosis treatment, BMD "is a common surrogate marker of osteoporosis treatment efficacy. However, due to the relatively small effect of treatment relative to the precision of the test, it is not practical to repeat BMD at intervals shorter than two years. Also, fracture risk reduction on treatment is far greater than would be predicted by the BMD increase achieved. Fewer than half of patients prescribed a bisphosphonate are taking the medication after 1 year. For these reasons, it is helpful to assess the effects of, and compliance with, treatment within a few months. Some

studies show improved adherence to treatment when turnover marker results were provided to patients, although this finding is not universal" (Coates, 2013).

Overall, the RACGP's guideline for osteoporosis management recognizes "the response of bone turnover markers to treatment, particularly in the first few months after initiating bisphosphonates or teriparatide, but does not yet recommend their routine use" (Coates, 2013).

Paget's Association, Guideline Development Group

This Guideline Development Group published a guideline titled "Diagnosis and Management of Paget's Disease of Bone in Adults". The relevant remarks include (Ralston et al., 2019):

- "Serum total ALP [total alkaline phosphatase] is widely available and considerably cheaper than other biochemical markers that have been assessed in PDB [Paget's Disease of Bone]."
- "If total ALP values are normal and clinical suspicion of metabolically active PDB is high, measurement of BALP, PINP, or uNTX may be considered to screen for metabolically active disease."
- "…elevations in markers of bone turnover occur in many disease states and cannot be used in isolation for the diagnosis of PDB."
- "Measurement of PINP is recommended to predict lesion extent, as defined by scintigraphy, after bisphosphonate therapy."
- "Measurement of biochemical markers of bone turnover are not recommended a means of predicting the response of bone pain to osteoclast inhibitors in PDB" (Ralston et al., 2019).

Royal Osteoporosis Society (ROS)

In 2021, the ROS updated a 2018 a statement on the use of bone markers and osteoporosis. In it, they included three reasons as to why bone markers may be used (Royal Osteoporosis Society, 2021):

> "a) To measure bone turnover as part of an assessment of bone strength and fracture risk. There haven't been many research trials to prove how effective this is, so other methods are usually used to assess bone strength, including a bone density scan to measure your bone density, along with your other risk factors.
> b) To monitor the effectiveness of osteoporosis drug treatments. Most treatments work by slowing the rate of bone resorption. The rate of bone formation also slows, but the overall effect is that the two processes come back into balance, leading to improved bone strength. The effect of a drug treatment on bone turnover can be assessed using bone markers within six months of starting treatment.
> c) In research trials, to assess osteoporosis drugs in development. Although there is evidence to suggest the value of bone marker tests as outlined above, expert opinion is divided on how useful or necessary they are, and further research is required to establish how they should be best used in the management of osteoporosis."

As to the recommendation on the use of bone markers, they noted that a "UK independent review in 2014, which looked at whether bone markers should be used to see if a drug treatment is working,

concluded there was insufficient evidence available to make recommendations. International expert guidance, however, says that although more research is needed, bone markers can be useful in some situations" (Royal Osteoporosis Society, 2021).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Several tests for bone turnover markers have been cleared by the U.S. Food and Drug Administration (FDA) using the 510(k) process including the collagen cross-links tests; pyrilinks test from Metra Biosystems which measures collagen type 1 cross-link, pyridium, Osteomark test from Ostex International which measures cross-linked N-telopeptides of type 1 collagen (NTx), and Serum Crosslaps One-step ELISA test which measures hydroxyproline. Other bone turnover cleared through the FDA 510(k) process tests include: Ostase from Beckman Coulter which measures bone-specific alkaline phosphatase (B-ALP), N-MID Osteocalcin One-step ELISA from Osteometer Bio Tech which measures osteocalcin (OC), and Elecsys® N-MID Osteocalcin Immunoassay (Roche Diagnostics).

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
82523	Collagen cross links, any method
83500	Hydroxyproline; free
83505	Hydroxyproline; total
83937	Osteocalcin (bone g1a protein)
84080	Phosphatase, alkaline; isoenzymes

Procedure codes appearing in 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
11/12/21	Altered requirements contained in coverage criteria #2 by removing "and efficacy" from this guideline.
2/13/23	Modified wording and formatting in overall criteria, and added Note 1 as reference for listing of bone markers.
1/8/24	The following changes were implemented: Removed previous coverage criteria #1; modified previous coverage criteria #2, now coverage criteria #1, to address frequency of testing in individuals receiving bisphosphonate treatment: "For individuals treated with bisphosphonates, measurement of bone turnover markers to assess an individual's compliance with bisphosphonate therapy **MEETS COVERAGE CRITERIA** at the following intervals: a) To establish baseline levels before initiating bisphosphonate treatment, b) Every three months after initiation or change of therapy for the first year, c) Every two years when no medication changes have occurred."

Biomarkers for Myocardial Infarction and Chronic Heart Failure

Policy #: AHS – G2150	**Prior Policy Name & Number (as applicable):** Cardiac Biomarkers for Myocardial Infarction (AHS-G2150)
Implementation Date: 9/15/21	**Date of Last Revision:** 10/26/23, 1/8/24 (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

Cardiac biomarkers are the biochemical markers released in blood from injured myocardial tissue in both acute and chronic conditions, such as myocardial infarction or heart failure. They become elevated in blood after a certain period and can be measured. Examples of cardiac biomarkers commonly used in the clinical setting include troponin and creatine kinase MB isoenzyme (CKMB) (Thygesen et al., 2007). Others, such as Suppression of tumorigenicity 2 (ST2), can serve in long-term as markers of cardiomyocyte stress and fibrosis for risk stratification of patients with a wide spectrum of cardiovascular diseases (Bayes-Genis et al., 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. For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of the following cardiac biomarkers for the diagnosis and/or prognosis of **MEETS COVERAGE CRITERIA** up to four times within the first 72 hours following initial presentation.

2. For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of the following cardiac biomarkers for the diagnosis and/or prognosis of MI **DOES NOT MEET COVERAGE CRITERIA**:

   a) Aspartate aminotransferase (AST/SGOT)
   b) Cardiac creatine kinase isoenzyme MB (CKMB)
   c) Creatine kinase (CK)
   d) Creatine kinase isoenzymes
   e) Lactate dehydrogenase (LD, LDH)
   f) Myoglobin

3. Measurement of B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) **MEETS COVERAGE CRITERIA** in any of the following situations:
   a) To diagnose heart failure in individuals presenting with dyspnea.
   b) To establish disease severity in individuals with chronic heart failure (up to four times per year in the outpatient setting).

4. Measurement of cardiac biomarkers in patients presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of cardiac biomarkers in an outpatient setting which is not capable of performing adequate clinical MI evaluation (e.g., independent lab or physician's office) **DOES NOT MEET COVERAGE CRITERIA**.

5. In the outpatient setting, qualitative measurement of cardiac troponin (troponin T or I) **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.*

6. For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of the following cardiac biomarkers for the diagnosis and/or prognosis of MI **DOES NOT MEET COVERAGE CRITERIA**:

   a) Copeptin
   b) Troponin C
   c) C-reactive protein
   d) Heart-type fatty acid binding protein (H-FABP)
   e) any other cardiac biomarkers not listed above

7. For all situations in the outpatient setting, analysis of ST2 and/or its isoforms (e.g., Presage ST2) **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: *Acute Coronary Syndrome/Myocardial Infarction Common Signs and Symptoms (Reeder, 2022):*

- Ischemic chest pain with radiation to an upper extremity, radiation to both arms, and pain associated with diaphoresis or with nausea and vomiting.
- Squeezing, tightness, pressure, constriction, crushing, strangling, burning, heartburn, fullness in the chest, band-like sensation, knot in the center of the chest, lump in throat, ache, heavy weight on chest and toothache (when there is radiation to the lower jaw).
- Ischemic pain often radiates to other parts of the body including the upper abdomen (epigastrium), shoulders, arms (upper and forearm), wrist, fingers, neck and throat, lower jaw and teeth (but not upper jaw), and not infrequently to the back (specifically the interscapular region).
- Shortness of breath, belching, nausea, indigestion, vomiting, diaphoresis, dizziness, lightheadedness, clamminess, and fatigue.

Atypical Signs and Symptoms (Reeder, 2022):

Dyspnea alone, weakness, nausea and/or vomiting, epigastric pain or discomfort, palpitations, syncope, or cardiac arrest.

III. Scientific Background

Acute coronary syndromes (ACS) represent continuous events starting with angina, reversible injury, progressing to unstable angina, these syndromes are frequently associated with minor myocardial damage, and myocardial infarction (MI) that results in extensive tissue necrosis (Thygesen et al., 2007). Patients with ACS usually present with chest pain and associated signs and symptoms. These patients are subdivided into two major categories based on the 12-lead electrocardiogram (ECG). If an ST-segment elevation is observed on the ECG, it is indicative of acute ST-elevation myocardial infarction (STEMI) type of ACS. If the ECG shows ST-segment depression, T-wave changes, or no ECG abnormalities, it is indicative of non-ST elevation myocardial infarction (NSTEMI) and unstable angina. ACS is complex. However, the most common cause is atherosclerotic coronary artery disease with rupture of atherosclerotic plaque (Amsterdam et al., 2014). The first documented definition of acute MI was established in 1979 by the World Health Organization (WHO). It included in the criteria for MI diagnosis the recommendation to use the rise or fall patterns of cardiac biomarkers, such as creatine kinase (CK), creatine kinase's MB isoenzyme (CK-MB), lactate dehydrogenase (LDH) or aspartate aminotransferase (AST) activities (WHO, 1979). Since then, other societies have proposed their own criteria for diagnosis. The third universal definition of MI includes typical clinical symptoms, suggestive ECG changes, or imaging evidence of new loss of viable myocardium or new regional wall abnormality with a rise and/or fall of cardiac biomarkers (Thygesen et al., 2012). Nonetheless, the universal criteria are being refined by cardiovascular societies and will likely change with scientific progress and better understanding of MI pathophysiology.

Myocardial infarction results in cardiac injury and extensive tissue necrosis. The cellular membranes become compromised and release structural proteins and other macromolecules into cardiac interstitial space. These release markers are called cardiac biomarkers. The levels of these cardiac biomarkers in blood will rise and fall with time after MI (Thygesen et al., 2007). The first cardiac biomarker, aspartate

aminotransferase (AST), was used for MI diagnosis in 1954. AST is present in human tissues as two isoenzymes: cytoplasmic and mitochondrial. AST is a non-specific biomarker and its activity could also be elevated in other conditions, such as hepatic congestion secondary to congestive heart failure. Since then, other cardiac biomarkers were used as an aid in diagnosis of MI, but due to their non-specificity and other reasons, many of them are no longer used in clinical practice or their use remains very limited (Danese & Montagnana, 2016). The most common cardiac biomarkers and their characteristics are summarized in the table from
(Danese & Montagnana, 2016):

Table 1 Characteristics of AMI biomarkers

Biomarker	First assay development, year	Molecular weight, Da	Kinetics			Sensitivity for myocyte necrosis	Specificity for myocyte necrosis
			First detection, hours	Maximum value, hours	Return to normal values, days		
AST	1954	105,000	3–4	15–28	5	++	+
LDH	1955	140,000	5–10	60–144	12	++	+
CK total enzyme activity	1960	83,000	3–9	10–20	3	++	+
CK-MB activity	1972	83,000	3–8	10–20	3	++	++
Myoglobin	1978	17,800	1–3	4–7	1–1.5	+++	+
CK-MB mass	1985	83,000	3–12	12–18	2–3	+++	+++
cTnI	1987	23,900	3–7	10–20	10	++++	++++
cTnT	1989	37,000	3–8	15–120	14	++++	++++

AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase MB isoenzyme; cTnI, cardiac troponin I; cTnT, cardiac troponin T.

Heart failure (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 (Colucci, 2022). Most patients with HF have symptoms due to impaired left ventricular (LV) myocardial function (Colucci & Dunlay, 2022; 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 (Colucci & Dunlay, 2022). Heart failure is often a progressive condition, beginning with predisposing factors and leading to the development and worsening of clinical illness (Colucci, 2021; Colucci & Dunlay, 2022).

Lactate Dehydrogenase (LDH, also known as LD)

Lactate dehydrogenase is a cytoplasmic enzyme present in many different tissues, such as skeletal muscle, liver, heart, kidney, and red blood cells. Five isoenzymes have been identified by gel electrophoresis and other techniques (Marshall et al., 1991). The heart isoenzymes, LD1 and LD2, have activity increases in blood five to ten hours after MI symptoms onset and remains elevated for up to ten

days (Danese & Montagnana, 2016). LD has poor specificity for cardiac tissue and is generally not recommended as a biomarker for the diagnosis of MI (Amsterdam et al., 2014; Jaffe & Morrow, 2019).

Myoglobin

Myoglobin is an oxygen-binding, cytoplasmic, heme protein. It is one of the first cardiac biomarkers measurable in the serum that appears between one and three hours after MI symptoms onset. Myoglobin is present in skeletal and cardiac muscles and is cleared by the kidneys (Vaidya, 1994). Its clinical utility is limited by its poor specificity. The main reason of using myoglobin in a clinical setting was its sensitivity for MI (Danese & Montagnana, 2016); but with appearance of sensitive troponin assays, myoglobin use offers little advantage for the diagnosis of MI (Eggers et al., 2004; Kavsak et al., 2007). Currently, there are no recommendations for myoglobin to be used in the diagnosis of MI (Amsterdam et al., 2014), and its use as cardiac biomarker is discouraged (Jaffe & Morrow, 2017).

Creatine Kinase (CK) Isoenzymes and Isoform MB (CKMB)

The cytosolic enzyme creatine kinase (CK), formerly known as creatine phosphokinase (Danese & Montagnana, 2016), is present as three cytosolic isoenzymes and one mitochondrial isoenzyme. These isoenzymes are dimers of M (muscle) and B (brain) chains that exist in three combinations: MM, MB and BB (Bessman & Carpenter, 1985). The CKMM is predominant in both heart and skeletal muscle, but CKMB is more specific for the myocardium. The total CK activity could be detected in blood 3-9 hours after MI, but it reaches the maximum levels in blood in 10-20 hours and returns to normal in about 72 hours (Penttila, Penttila, & Rantanen, 2000). The measurement of total CK activity is not specific to MI because it also increases in liver, biliary tract, kidneys, and skeletal muscle disease, and its measurement is problematic in older individuals with lower muscle mass (Dillon et al., 1982; Heller, Blaustein, & Wei, 1983; Yusuf et al., 1987). CKMB mass (CKMB protein concentration measurements) was once the cardiac biomarker of the choice that replaced CK, CKMB activity, AST, and LDH (Danese & Montagnana, 2016). However, with arrival of cardiac troponin assays, the use of CKMB became less popular. Some clinicians advocate for the use of CKMB for diagnosis and prognosis of MI, but cardiac troponins have shown either equally reliable or superior results compared to CKMB; consequently, troponin is the recommended test for MI diagnosis now (Amsterdam et al., 2014; Jaffe & Morrow, 2017).

Engel and Rockson (2020) studied the use of CK-MB in early diagnosis of myocardial infarction within the first nine hours of the hospital stay. The authors studied 528 patient charts of patients who had complained of chest pain within the past year were studied. An enzymatic diagnosis was assigned if CK-MB exceeded the normal values. The diagnosis of each patient before 9 hours (early diagnosis) was compared to the ultimate diagnosis at 14-24 hours (final diagnosis). Of the 528 patients, 195 (36.9%) had an early MI diagnosis within 9 hours and 190 patients (97.4%) of these did have an ultimate diagnosis of MI. Therefore, the authors conclude that "standard CK-MB measurements within 9 hours of arrival provided an accurate clinical assessment in > 99% of the cases (Engel & Rockson, 2020)."

Troponins

Cardiac Biomarkers for Myocardial Infarction, continued

The regulatory protein troponin in the troponin complex is composed of three isoforms. Troponin C (TnC) is responsible for calcium binding and has no role to play as a cardiac biomarker. Troponin I (TnI) and Troponin T (TnT) are responsible for inhibition of ATPase activity and tropomyosin binding, respectively (Greaser & Gergely, 1971). Contrary to all previously used cardiac biomarkers, cardiac troponins have high specificity for cardiac tissue. The cardiac troponins have a specific pattern of expression because they have different amino sequences encoded by different genes for skeletal and cardiac muscles. Cardiac TnI has an additional 31-amino acid residue compared to skeletal muscle. This protein is not expressed in normal, regenerating, or diseased skeletal muscle from human or animal origin (Bodor, Porterfield, Voss, Smith, & Apple, 1995). Cardiac TnT has an additional 11-amino acid residue, but this protein was also found in regenerating rat skeletal muscle, during human fetal development, and in diseased human skeletal muscle (Anderson, Malouf, Oakeley, Pagani, & Allen, 1991; Bodor et al., 1997; Saggin, Gorza, Ausoni, & Schiaffino, 1990). In addition, cardiac TnT was also found in skeletal muscle specimens from patients with muscular dystrophy, polymyositis, and chronic renal disease (Bodor et al., 1997; McLaurin, Apple, Voss, Herzog, & Sharkey, 1997).

Neumann et al. (2019) evaluated high-sensitivity troponin (troponin I and T)'s ability to predict myocardial infarction and subsequent 30-day outcomes. The authors developed a risk assessment tool based on patients presenting to the emergency department with "symptoms suggestive of myocardial infarction". Concentrations of troponin I or T were measured at presentation and after early or late serial sampling. Cutoffs were then determined to create cutoffs for risk assessment. Among the 22651 patients (9604 in derivation cohort, 13047 in validation cohort), the total prevalence of myocardial infarction was 15.3%. The authors found that "lower high-sensitivity troponin concentrations at presentation and smaller absolute changes during serial sampling were associated with a lower likelihood of myocardial infarction and a lower short-term risk of cardiovascular events" (Neumann et al., 2019).

Anand et al. (2019) evaluated the adoption rate of the universal definition of myocardial infarction and the corresponding recommendations. A total of 1902 medical centers over 23 countries were surveyed, and the authors obtained answers regarding the primary biomarker, diagnostic thresholds, and clinical pathways used to identify myocardial infarction. The authors found that cardiac troponin was the primary biomarker used at 96% of surveyed sites, with 41% of these sites using high-sensitivity troponins. The sites using high-sensitivity assays were also more likely to use serial sampling (91% vs 78% using "contemporary" sensitivity troponin) and the 99% percentile diagnostic threshold (74% vs 66%). Use of creatine kinase-MB (CKMB) was "very limited" outside of Latin America (Anand, Shah, Beshiri, Jaffe, & Mills, 2019).

In addition, other cardiac biomarkers, such as heart-type fatty acid binding protein (H-FABP) and copeptin, have been reported in the scientific literature. However, they are not commonly used in clinical settings (Jaffe & Morrow, 2017).

Boeddinghaus et al. (2020) compared the diagnostic accuracy of high-sensitivity cardiac troponin (hs-cTn) TriageTrue assay in patients with suspected myocardial infarction (MI) with other laboratory assays including f hs-cTnTElecsys assay and hs-cTnI-Architect assay. A total of 1,261 patients with symptoms

suggestive of MI were enrolled in the study. The TriageTrue assay ruled out patients with troponin I concentration < 3 ng/l and classified these patients as low risk of MI and ruled in patients with a troponin I concentration > 60 ng/l. Out of the 1,261 patients enrolled in the study, 178 were diagnosed with MI based on troponin I levels of > 60 ng/l using the TriageTrue assay. TriageTrue troponin I concentrations were higher in patients with MI than in patients with other final diagnoses. Other diagnosis included unstable angina in 13 of 1,261 (9%), tachyarrhythmia, Takotsubo syndrome, heart failure, or myocarditis in 208 patients (17%), and noncardiac symptoms in 714 patients (57%). The AUC of the TriageTrue assay was 0.95, the hs-cTnT-Elecsys assay AUC was 0.93, and hs-cTnI-Architect assay AUC was 0.92. The TriageTrue algorithm allowed providers to make a triage decision after one hour in 401 of 545 patients. The efficacy for rule-out or rule-in was 43% for the TriageTrue, 25% in f hs-cTnTElecsys, and 22% in hs-cTnI-Architect. Ruled-out patients had cumulative event rates of 0% at 30 days and 1.6% at 2 years. Overall, the authors conclude that "POC-hs-cTnI-TriageTrue assay provides high diagnostic accuracy in patients with suspected MI with a clinical performance that is at least comparable to that of best-validated central laboratory assays (Boeddinghaus et al., 2020)."

Heart-type fatty acid binding protein (H-FABP)

H-FABP, a small cytoplasmic protein present in cardiomyocytes, is believed to have a function in myocardial lipid homeostasis (Glatz & van der Vusse, 1990). Because of its small size, this protein appears in the blood after MI almost as early as myoglobin, but it has better specificity than myoglobin for cardiac tissue (Van Nieuwenhoven et al., 1995). Seino et al compared the use of H-FABP with rapid troponin in 371 patients with acute chest pain (Seino et al., 2003). Their study demonstrated that H-FABP had significantly higher sensitivity (89%) than troponin T (22%) and myoglobin (38%), but it has lower specificity (52%) than troponin (94%). Other studies were performed to compare H-FABP to troponins; however, they were unable to demonstrate superior results compared to troponins. H-FABP is not encouraged for assessment of MI as troponins are generally superior (Jaffe & Morrow, 2021).

In a prospective, cross-sectional study, Nguyen et al. (2020) studied the diagnostic utility of H-FABP in the early diagnosis of acute MI in comparison with troponin I and CK-MB. 216 patients enrolled in the study with 179 of those diagnosed with acute MI. H-FABP, CK-MB, and troponin I levels were compared. H-FABP reached its highest concentration in 6-12 hours after symptoms of chest pain, with a mean value of 169 ng/mL in acute MI patients. The cut-off value was 5.7 ng/ml with 90.5% sensitivity and 100% specificity. The combination of H-FABP, CK-MB and troponin I together had the highest sensitivity of 97.2%. The AUC of H-FABP was observed to be 0.99, which was higher than CK-MB (0.92) and troponin I (0.86). The authors conclude that "H-FABP can be used as a reliable diagnostic cardiac biomarker in the early detection of AMI for patients who came to the emergency room within 12h of onset of chest pain (Nguyen et al., 2020)."

Copeptin

Copeptin is the 39 amino acid C-terminal fragment cleaved from pro-arginine vasopressin (AVP). After MI, copeptin levels increase rapidly and decline over the next two to five days (Khan et al., 2007). In the Copeptin Helps in the Early Detection of Patients With Acute Myocardial Infarction (CHOPIN) 16-site study involving 1,967 patients presenting within 6 hours of pain onset, copeptin was shown to have a

potential value in ruling out MI with a negative predictive value greater than 99% when combined with TnI measurements (Maisel et al., 2013). The Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) multicenter study, involving 1,439 patients presenting with MI symptoms, demonstrated no benefit in using copeptin as a an early rule-out cardiac biomarker for MI (Hillinger et al., 2015). Copeptin is not encouraged for assessment of MI as troponins are generally superior (Jaffe & Morrow, 2021).

ST2

Part of the interleukin-1 receptor family with two isoforms, ST2 has 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 et al., 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).

By-type Natriuretic Peptide (BNP)

B-type natriuretic peptide (BNP) plays a role in salt and water management as well as pressure regulation within the natriuretic peptide system. When the prohormone proBNP is cleaved, it produces BNP and N-terminal pro-BNP; BNP is released mostly from the left ventricle in the heart. An increase in the release of BNP may be indicative of heart failure and rapid measurement can establish or exclude the diagnosis of heart failure in patients with acute dyspnea.

A number of clinical assays are available for plasma BNP. These range from rapid point-of-care tests to lab tests that provide precise values for BNP. An NT-proBNP concentration greater than 900 pm/mL is "roughly" the same as a BNP concentration that is greater than 100 pg/mL (Wilson S Colucci, 2023).

Natriuretic peptide biomarkers should be measured in patients who present with dyspnea to diagnose heart failure, but these biomarkers must be considered as part of a complete patient evaluation and not used in isolation. For prognosis, natriuretic peptide biomarkers can be used in patients with chronic HF and used when patients are admitted to the hospital with acutely decompensated HF. Lastly, there may be value in measuring natriuretic peptide biomarkers predischarge from the hospital (Wilson S Colucci, 2023).

N-terminal pro-B-type Natriuretic Peptide (NT-proBNP)

Measurement of NT-proBNP is of value in diagnosis and prognosis of heart failure and other cardiovascular diseases. Studies show that the accuracy of diagnosing heart failure across various settings improves with measurement of NT-proBNP values. Like BNP, NT-proBNP is helpful when used with patients presenting with dyspnea. The "optimal" measurement value for differentiating between heart failure and other causes of dyspnea varies with patient age.

BNP and N-terminal pro-BNP both fall in concentration after effective therapeutic treatment of chronic heart failure, which means that serial measurements have shown some promise in therapeutic management. However the effectiveness and use of serial BNP measurements in monitoring patient response to acute heart failure treatment is still under investigation (Wilson S Colucci, 2023).

Proprietary Testing

Proprietary tests for various biomarkers are available in several clinical settings. Platforms including Roche's "CARDIAC Trop T Sensitive test" and Responsebio's battery of cardiac tests emphasize their speed (on the scale of minutes) and versatility (ResponseBio, 2020; Roche, 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).

Clinical Utility and Validity

Jeong et al. (2020) studied the diagnostic value of copeptin for early diagnosis of acute MI in comparison with troponin I and CK-MB. 271 patients complaining of chest pain within 6 hours of onset were studied within the emergency department. The diagnostic performance of copeptin, troponin I, and CK-MB was compared by assessing the AUC and ROC curve analysis. After comparing AUC, copeptin had a significantly better diagnosis value than troponin I in patients with chest pain within two hours of onset. In addition, troponin I and copeptin together had better diagnostic performance than CK-MB and troponin I combination. Overall, the authors conclude that "the combination of troponin I and copeptin improves AMI diagnostic performance in patients with early-onset chest pain in an ED setting (Jeong et al., 2020)."

Ky et al. (2011) 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).

Wang et al. (2012) 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).

Wang et al. (2018) investigated the possibility of using sST2 as a biomarker to distinguish between acute aortic dissection and other causes of acute chest pain. Using an R&D Systems assay to measure plasma concentrations of sST2 in 1360 patients with a cutoff of 34.6 ng/mL, the researchers found that "sST2 had a sensitivity of 99.1%, specificity of 84.9%, positive predictive value of 68.7%, negative predictive value of 99.7%, positive likelihood ratio of 6.6, and negative likelihood ratio of 0.01." Additionally, within 24 hours of symptom onset, sST2 levels were higher in those with acute aortic dissection in comparison to those with acute myocardial infarction or pulmonary embolism. sST2 was also superior in overall diagnostic performance to D-dimer and troponin I, using the area under receiver operating characteristic curves.

Wijk et al. (2014) provided a follow-up on the largest study of long-term results of intensified NT-proBNP-guided versus symptom-guided management of elderly patients with heart failure. The TIME-CHF study randomized 499 patients with heart failure that were ages 60 and older with left ventricular ejection fraction; patients were provided either guided NT-proBNP treatment or symptom-guided therapy over a period of 18 months. The results of the study showed "NT-proBNP–guided therapy did not improve the primary end point compared with symptom-guided therapy but did improve HF hospitalization-free survival" (Wijk et al., 2014).

Januzzi et al. (2013) 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. (2018) 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 et al., 2018).

Dimitropoulos et al. (2020) 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. (2020) 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).

IV. Guidelines and Recommendations

2018 ESC/ACC/AHA/WHF Fourth Universal Definition of Myocardial Infarction

Both cTnI and cTnT are recommended for evaluation of myocardial injury, and high-sensitivity cTn assays are recommended for routine clinical use. An acute MI is designated when a rising/falling pattern is seen with cTn levels and if there is at least one measurement greater than the 99th percentile of the upper reference limit (URL) (Jaffe et al., 2018).

CKMB is considered less sensitive and specific than either troponin. However, in the absence of a cTn assay, CK-MB is considered the best alternative. A measurement of CK-MB above the 99th percentile of the URL should be "designated as the decision level for the diagnosis of MI". Sex-specific CK-MB values should be used (Jaffe et al., 2018).

In the 2019 AHA guideline discussing the "Contemporary Diagnosis and Management of Patients With Myocardial Infarction in the Absence of Obstructive Coronary Artery Disease [MINOCA]", the AHA notes that the diagnostic criteria of MINOCA follows the "Fourth Universal Definition of Myocardial Infarction" above, specifically the rise or fall of cardiac troponin levels with at least one value above the 99th percentile of the reference limit. The guideline considers this definition "fundamental" to identifying and defining MINOCA (Tamis-Holland Jacqueline et al., 2019).

2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes (NSTE-ACS)

The American College of Cardiology (ACC) and the American Heart Association (AHA) have developed clinical practice guidelines to provide recommendations applicable to patients with or at risk of developing cardiovascular disease and to provide guidance to clinicians on optimal management of patients with NSTE-ACS. In their comprehensive document, the AHA/ACC panel has provided recommendations for initial evaluation and management of patients presenting with ACS symptoms, for the early hospital care, myocardial revascularization, late hospital care, hospital discharge and posthospital discharge care, special patient groups and quality of care and outcomes for ACS. The Task

Biomarkers for Myocardial Infarction and Chronic Heart Failure, continued

Force recommended to stratify patients with suspected ACS based on the likelihood of ACS and those with high-risk features should be referred immediately to the emergency department (ED). They have provided specific recommendations for the use of cardiac biomarkers in the diagnosis and prognosis of MI. They specifically recommended using troponin (troponin I or T when contemporary assay is used) for the diagnosis of MI. According to AHA/ACC guidelines, the cardiac troponin is recommended and should be measured at presentation and 3 to 6 hours after symptom onset in all patients who present with ACS symptoms. The panelists recommended identifying rising and/or falling pattern of troponin. In addition, they recommended measuring troponin levels beyond 6 hours after symptom onset in patients with normal troponins on serial examination when ECG changes and/or clinical presentation suggests ACS. If the onset of symptoms is not clearly identified, they recommended using the time of presentation as the time of onset for measuring troponin. The AHA/ACC guideline clearly highlighted that CKMB or myoglobin should not be used for the diagnosis of ACS. All recommendations for the use of cardiac biomarkers in the diagnosis of MI were level A evidence.

The AHA/ACC guideline considered all recommendations in the use of cardiac biomarkers for ACS prognosis as level of evidence B. They considered the presence and magnitude of troponin elevations useful for short- and long-term prognosis. The re-measurement of troponin once on day 3 or day 4 in patients with MI was considered reasonable to estimate the infarct size and dynamics of necrosis. Finally, they considered the use of B-type natriuretic peptide to be reasonable for additional prognostic information.

The recommendations for the use of cardiac biomarkers in the diagnosis and prognosis of MI was well summarized in Table from 2014 AHA/ACC guidelines p.2655 (Amsterdam et al., 2014):

TABLE 5 Summary of Recommendations for Cardiac Biomarkers and the Universal Definition of MI

Recommendations	COR	LOE
Diagnosis		
Measure cardiac-specific troponin (troponin I or T) at presentation and 3–6 h after symptom onset in all patients with suspected ACS to identify pattern of values	I	A
Obtain additional troponin levels beyond 6 h in patients with initial normal serial troponins with electrocardiographic changes and/or intermediate/high risk clinical features	I	A
Consider time of presentation the time of onset with ambiguous symptom onset for assessing troponin values	I	A
With contemporary troponin assays, CK-MB and myoglobin are not useful for diagnosis of ACS	III: No Benefit	A
Prognosis		
Troponin elevations are useful for short- and long-term prognosis	I	B
Remeasurement of troponin value once on d 3 or 4 in patients with MI may be reasonable as an index of infarct size and dynamics of necrosis	IIb	B
BNP may be reasonable for additional prognostic information	IIb	B

ACS indicates acute coronary syndromes; BNP, B-type natriuretic peptide; CK-MB, creatine kinase myocardial isoenzyme; COR, Class of Recommendation; LOE, Level of Evidence; and MI, myocardial infarction.

2013 (published 2014) Society for Cardiovascular Angiography and Interventions (SCAI)

In their expert consensus document titled "Consideration of a New Definition of Clinically Relevant Myocardial Infarction After Coronary Revascularization," the SCAI expert panel introduced a new definition of clinically relevant MI after coronary revascularization percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). In their definition of clinically relevant MI after both PCI and CABG procedures, authors gave recommendations according to 3 different types of clinical presentation. In the first case, when patient has a normal CKMB baseline: "The peak CK-MB measured within 48 hours of the procedure rises to ≥10x the local laboratory ULN, or to ≥5x ULN with new pathologic Q-waves in ≥2 contiguous leads or new persistent LBBB, OR in the absence of CK-MB measurements and a normal baseline cTn, a cTn (I or T) level measured within 48 hours of the PCI rises to ≥70x the local laboratory ULN, or ≥35x ULN with new pathologic Q-waves in ≥2 contiguous leads or new persistent LBBB". In the case when patients have elevated baseline CKMB (or cTn) with stable of falling biomarkers levels, they issued the following recommendation: "The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above from the most recent pre-procedure level". And, in patients with elevated CKMB (or cTn), but without stable or falling biomarkers level, the recommendation was: "The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above plus new ST-segment elevation or depression plus signs consistent with a clinically relevant MI, such as new onset or worsening heart failure or sustained hypotension". The authors have expressed preference to use CKMB instead of cTn, but they have included cTn in their definition if CKMB was not available (Moussa et al., 2013).

2015 AHA Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care

In their review of previously issued guidelines, the expert panel introduced new recommendations for diagnostic interventions in ACS regarding cardiac biomarkers. They still recommended to use Troponin in following situations: "We recommend against using hs-cTnT and cTnI alone measured at 0 and 2 hours (without performing clinical risk stratification) to identify patients at low risk for ACS (Class III: Harm, LOE B-NR). We recommend that hs-cTnI measurements that are less than the 99th percentile, measured at 0 and 2 hours, may be used together with low-risk stratification (TIMI score of 0 or 1 or low risk per Vancouver rule) to predict a less than 1% chance of 30-day MACE (Class IIa, LOE B-NR). We recommend that negative cTnI or cTnT measurements at 0 and between 3 and 6 hours may be used together with very low-risk stratification (TIMI score of 0, low-risk score per Vancouver rule, North American Chest Pain score of 0 and age less than 50 years, or low-risk HEART score) to predict a less than 1% chance of 30-day MACE (Class IIa, LOE B-NR)". They did not express a preference in cardiac biomarkers to use, nor did they gave any recommendations regarding CKMB (O'Connor Robert et al., 2015).

American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA)

In 2017, the ACC/AHA/HFSA included information on BNP and NT-proBNP measurement for establishing prognosis or disease severity in chronic HF. Their recommendations:

- For prevention:
  - "Class IIa recommendation (Level of Evidence: B-R) for utilizing natriuretic peptide biomarker-based screening for those at risk of developing HF, followed by team-based care including a

cardiovascular specialist optimizing guideline-directed medical therapy (GDMT), to prevent the development of left ventricular dysfunction (systolic or diastolic) or new-onset HF.z"

- For diagnosis:
  - "Class I recommendation (Level of Evidence: A) for measurement of natriuretic peptide biomarkers in patients presenting with dyspnea, to support a diagnosis or exclusion of HF."
- For prognosis or added risk stratification:
  - "Class I recommendation (Level of Evidence: A) for measurement of B-type natriuretic peptide (BNP) or N-terminal (NT)-proBNP for establishing prognosis or disease severity in chronic HF."
  - "Class I recommendation (Level of Evidence: A) for measurement of baseline natriuretic peptide biomarkers and/or cardiac troponin on admission to the hospital to establish a prognosis in acutely decompensated HF."
  - "Class IIa recommendation (Level of Evidence: B-NR) for measurement of a predischarge natriuretic peptide level during a HF hospitalization, to establish a post-discharge prognosis."
  - "Class IIa recommendation (Level of Evidence: B-NR) for measurement of other clinically available tests, such as biomarkers of myocardial injury or fibrosis, in patients with chronic HF for additive risk stratification (Yancy et al., 2017)."

The full ACC/AHA article does not appear to support a standard of care that includes measuring BNP/NT-BNP for purposes of serial monitoring or therapeutic management, noting that "Because of the absence of clear and consistent evidence for improvement in mortality and cardiovascular outcomes, there are insufficient data to inform specific guideline recommendations related to natriuretic peptide-guided therapy or serial measurements of BNP or NT-proBNP levels for the purpose of reducing hospitalization or deaths in the present document" (Yancy et al., 2017).

In 2022, the ACC/AHA/HFSA updated their 2017 guideline on the management of heart failure. Regarding BNP and NT-proBNP assays, the authors emphasize that both tests can be used to establish the presence and severity of heart failure. However, they caveat that diagnostic sensitivity is impacted when a patient Is overweight – patients who are obese sometimes measure as having low levels of BNP and NT-proBNP.

Additional points of emphasis include:

- "A substantial evidence base supports the use of natriuretic peptide biomarkers for excluding HF as a cause of symptoms in ambulatory and emergency department settings."
- "Although a reduction in BNP and NT-pro-BNP has been associated with better outcomes, the evidence for treatment guidance using serial BNP or NT-proBNP measurements remains insufficient."
- "A widening array of biomarkers including markers of myocardial injury, inflammation, oxidative stress, vascular dysfunction, and matrix remodeling have been shown to provide incremental prognostic information over natriuretic peptides but remain without evidence of an incremental management benefit."

The recommendations for the use of biomarkers was summarized in the table provided by the ACC/AHA/HFSA (Heidenreich, 2022):

Biomarkers for Myocardial Infarction and Chronic Heart Failure, continued

Recommendations for Use of Biomarkers for Prevention, Initial Diagnosis, and Risk Stratification
Referenced studies that support the recommendations are summarized in the Online Data Supplements.

COR	LOE	RECOMMENDATIONS
1	A	1. In patients presenting with dyspnea, measurement of B-type natriuretic peptide (BNP) or N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) is useful to support a diagnosis or exclusion of HF (1-12).
1	A	2. In patients with chronic HF, measurements of BNP or NT-proBNP levels are recommended for risk stratification (11,13-29).
1	A	3. In patients hospitalized for HF, measurement of BNP or NT-proBNP levels at admission is recommended to establish prognosis (11,13-19).
2a	B-R	4. In patients at risk of developing HF, BNP or NT-proBNP-based screening followed by team-based care, including a cardiovascular specialist, can be useful to prevent the development of LV dysfunction or new-onset HF (30,31).
2a	B-NR	5. In patients hospitalized for HF, a predischarge BNP or NT-proBNP level can be useful to inform the trajectory of the patient and establish a postdischarge prognosis (14,17,20-29).

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 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).

European Society of Cardiology (ESC)

The ESC notes measurement of cardiac troponins as "mandatory" in all patients with suspected non-ST-elevation acute coronary syndromes. The guidelines assert that cardiac troponins are more sensitive and specific biomarkers of cardiomyocyte injury than CK, CKMB, and myoglobin. However, if troponin measurement is not possible, measurement of copeptin is recommended.

The ESC also acknowledges the natriuretic peptides (B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide and midregional pro-A-type natriuretic peptide) as providing useful prognostic information along with the troponins. The ESC mentions other biomarkers such as midregional pro-adrenomedullin, growth differentiation factor 15 and copeptin, but they cannot recommend them at this time as their added value in risk assessment seems "marginal" (Gencer et al., 2016).

The 2019 ESC guidelines focusing on chronic coronary syndromes states that for "clinical suspicion of coronary artery disease instability…management should follow the Guidelines for ACS without persistent ST-segment elevation", which is discussed above (Knuuti et al., 2019).

The 202O ESC guidelines focus on diagnosis of acute coronary syndrome. Regarding MI, they recommend that "the routine use of copeptin as an additional biomarker for the early rule-out of MI should be considered where hs-cTn assays are not available." In addition, "CK-MB shows a more rapid decline after MI and may provide added value for detection of early reinfarction (Collet et al., 2021)."

In their 2016 guidelines on acute and chronic heart failure, 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).

However, in a 2021 update, ESC (with special contribution from the Heart Failure Association) lists the key elements for HF and CMP diagnostic workups, and among the laboratory exams recommended under Table 26 for the "Initial diagnostic assessment in patients with suspected cardiomyopathy", they include the use of ST2:

"Laboratory exams including cardiac and muscular enzymes, liver and renal function, haemoglobin, white blood cell count (including differential white blood cell count to detect eosinophilia), natriuretic peptides, thyroid function tests, iron status, and markers of systemic auto-immune disease (hsCRP, anti-nuclear antibodies, soluble IL-2 receptor)" (McDonagh et al., 2021)

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 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).

ACC/AATS/AHA/ASE/ASNC/SCAI/ SCCT/STS 2016 Appropriate Use Criteria for Coronary Revascularization in Patients with Acute Coronary Syndromes Guidelines

In 2016 The American College of Cardiology (ACC), Society for Cardiovascular Angiography and Interventions (SCAI), Society of Thoracic Surgeons (STS), and American Association for Thoracic Surgery (AATS), along with key specialty and subspecialty societies created an Appropriate Use Task Force with the mission to revise the appropriate use criteria (AUC) for coronary revascularization. They have used clinical scenarios to mimic patient presentations seen in everyday clinical practice and included information on symptom status, presence of clinical instability or ongoing ischemic symptoms and other characteristics. They follow 2014 AHA/ACC recommendations for the use of cardiac biomarkers (Amsterdam et al., 2014).

American Society for Clinical Pathology (ASCP)

The ASCP recommends against testing CK-MB or myoglobin to diagnose an acute myocardial infarction. Instead, they recommend testing either troponin I or T. They also assert that both troponins are specific to cardiac injury and that there is much support for relying solely on troponin (ASCP, 2015).

National Institute for Health and Care Excellence (NICE)

NICE recommends diagnosis of MI using the "detection of rise and/or fall of cardiac biomarkers values [preferably cardiac troponin (cTn)] with at least one value above the 99th percentile of the upper reference limit and at least one of the following:

- symptoms of ischaemia
- new or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB)
- development of pathological Q waves in the ECG
- imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
- identification of an intracoronary thrombus by angiography" (NICE, 2016).

Currently, the 2018 NICE recommendations on chronic heart failure do not mention the usage of ST2 as a marker for diagnosing chronic heart failure. Instead, they recommend to "measure N-terminal pro-B-type natriuretic peptide (NT-proBNP) in people with suspected heart failure" (NICE, 2018).
In 2020, NICE released recommendations on the use of high sensitivity troponin tests to help rule out NSTEMI earlier in those presenting to an emergency department with chest pain and suspected acute coronary syndrome. NICE recommends the use of the following assays: Access High-Sensitivity Troponin I Assay, ADVIA Centaur High-Sensitivity Cardiac Troponin-I Assay, Alinity High Sensitive Troponin-I assay, ARCHITECT STAT High Sensitive Troponin-I assay, Atellica IM High-Sensitivity Cardiac Troponin I Assay, Dimension Vista High-Sensitivity Cardiac Troponin I Assay, Dimension EXL High-Sensitivity Cardiac Troponin I Assay, Elecsys Troponin T-high sensitive assay, Elecsys Troponin T-high sensitive STAT assay, VIDAS High sensitive Troponin I assay, and VITROS High Sensitivity Troponin I Assay. NICE mentions that although the "TriageTrue test has the potential to be cost effective, its diagnostic accuracy when used on whole blood is uncertain (NICE, 2020)." Regarding use of these assays, NICE recommends using a threshold at or near the limit of detection, which varies depending on the assay used. If this sample is positive, it should not be used to rule in NSTEMI. If taking multiple samples, take a sample at initial assessment followed by a second sample taken 30 minutes to 3 hours after. Use 99th percentile thresholds or thresholds at or near the limit of detection of the assay (NICE, 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
82550	Creatine kinase (CK), (CPK); total
82552	Creatine kinase (CK), (CPK); isoenzymes
82553	Creatine kinase (CK), (CPK); MB fraction only
82554	Creatine kinase (CK), (CPK); isoforms
82725	Fatty acids, nonesterified
83006	Growth stimulation expressed gene 2 (ST2, Interleukin 1 receptor like-1)
83615	Lactate dehydrogenase (LD), (LDH);
83625	Lactate dehydrogenase (LD), (LDH); isoenzymes, separation and quantitation
83874	Myoglobin
83880	Natriuretic peptide
84484	Troponin, quantitative
84512	Troponin, qualitative
84588	Vasopressin (antidiuretic hormone, ADH)
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.

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VIII. Revision History

Revision Date	Summary of Changes
10/26/23	The following changes were implemented: policy expanded to include information and coverage for the ST2 analysis/assay moved (previously in G2130); resulted in a title change to "Biomarkers for Myocardial Infarction and Chronic Heart Failure"; was previously titled ("Cardiac Biomarkers Myocardial Infarction"); moved repeat troponin testing from Note 1 into sub-criteria of coverage criteria #1, resulting in a reformatting of coverage criteria #1. Now reads: "For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), quantitative measurement of cardiac troponin (troponin T or I) for the diagnosis of myocardial infarction (MI) (when tested at an outpatient facility capable of performing an adequate clinical MI evaluation) **MEETS COVERAGE CRITERIA** up to four times within the first 72 hours following initial presentation."; new coverage criteria #4: "For all situations, qualitative measurement of cardiac troponin (troponin T or I) **DOES NOT MEET COVERAGE CRITERIA.**"; with policy expansion, addition of new coverage criteria #5: "5) For all situations in the outpatient setting, analysis of ST2 and/or its isoforms (e.g., Presage ST2) **DOES NOT MEET COVERAGE CRITERIA.**"
1/8/24	The following changes were implemented: added new coverage criteria #3: "Measurement of B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) **MEETS COVERAGE CRITERIA** in any of the following situations: a) To diagnose heart failure in individuals presenting with dyspnea. b) To establish disease severity in individuals with chronic heart failure (up to four times per year in the outpatient setting)."; edited coverage criteria #4 for clarity: "for all situations" changed to "In the outpatient setting."

Cardiovascular Disease Risk Assessment

Policy #: AHS – G2050	**Prior Policy Name & Number (as applicable):** • AHS – G2010 – Lipid Panels • AHS – G2050 – Novel Biomarkers in Risk Assessment and Management of Cardiovascular Disease • AHS – G2053 – Cardiovascular Risk Panels • AHS – G2106 – Measurement of Serum Intermediate Density Lipoproteins • AHS – M2082 – Measurement of Lipoprotein, Associated Phospholipase A2 • AHS – G2104 – Measurement of Long-Chain Omega-3 Fatty Acids • AHS – G2096 – Homocysteine Testing • AHS – M2090 – Genotyping for 9P21 Single Nucleotide Polymorphisms • AHS – M2102 – KIF6 Genotyping for Predicting Cardiovascular Risk and/or Effectiveness of Statin Therapy • AHS – M2064 – Genetic Expression to Predict Coronary Artery Disease
Implementation Date: 9/15/21	**Date of Last Revision:** 4/26/22, 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

Cardiovascular risk assessment comprises of the means and processes to predict the probability of developing a cardiovascular disease. These are a group of tests and health factors that have been proven to indicate a person's chance of having a cardiovascular event such as a heart attack or stroke. Tests typically used to assess cardiovascular risk include lipid profiles or panels, biomarkers, and cardiovascular risk panels. 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.

Cardiovascular Disease Risk Assessment, continued

1) For individuals 18 years of age or older, lipid panel testing (see Note 1) **MEETS COVERAGE CRITERIA** under any of the following conditions:

a) To screen for cardiovascular disease (CVD) risk:

i) Every 4 to 6 years for individuals ages 18 to 79 years.

ii) Annually for individuals at increased risk for cardiovascular disease (as defined by 2013 ACC/AHA Pooled Cohort Equations (PCEs) to calculate 10-year risk of CVD events [see Note 2]).

b) Annually for individuals at an increased risk of dyslipidemia due to any of the following conditions:

i) Obesity or metabolic syndrome

ii) Nephrotic syndrome

iii) Hypothyroidism

iv) Hyperthyroidism

v) Pancreatitis

vi) Diabetes

vii) Chronic kidney disease

viii) Cushing syndrome

ix) Pregnancy

x) Cholestatic liver disease

xi) Lipid metabolism disorders, such as Gaucher disease in adults

c) For individuals who are about to begin or who are currently receiving statin therapy, at the following intervals:

i) To establish baseline levels before initiating statin therapy.

ii) Every four to twelve weeks after initiation or change of therapy.

iii) Annually when no medication changes have occurred.

d) For individuals on a long-term drug therapy that requires lipid monitoring (e.g., Accutane, anti-psychotics).

e) For HIV positive individuals who are about to begin or who are currently receiving antiretroviral therapy (ART), at the following intervals:

i) To establish baseline levels before initiating ART.

ii) Every one to three months after initiation or change of therapy.

iii) Every six to twelve months when no medication changes have occurred.

2) Measurement of apolipoprotein B (apoB) **MEETS COVERAGE CRITERIA** for any of the following situations:

a) For individuals with hypertriglyceridemia.

b) For individuals with diabetes mellitus.

c) For individuals with obesity or metabolic syndrome.

d) For individuals with other dyslipidemias (such as very low LDL-C).

e) For individuals who are on lipid therapy.

f) For individuals who are suspected to have familial dysbetalipoproteinemia or familial combined hyperlipidemia.

3) For individuals 18 years of age or older, measurement of lipoprotein a (Lp(a)) once per lifetime **MEETS COVERAGE CRITERIA.**

4) For individuals for whom a risk-based treatment decision is uncertain (after quantitative risk assessment using ACC/AHA PCEs to calculate 10-year risk of CVD events [see Note 2]), testing for C-reactive protein with the high-sensitivity method (hs-CRP) **MEETS COVERAGE CRITERIA** at the following frequency:

a) For initial screening, two measurements at least two weeks apart.

b) If the initial screen was abnormal, follow-up screening is allowed up to once per year.

5) For CVD risk assessment, measurement of lipoprotein-associated phospholipase A2 (Lp-PLA2) **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 an individual's illness.

6) For all other cardiovascular disease risk assessments not described above, testing for CRP **DOES NOT MEET COVERAGE CRITERIA.**

7) For CVD risk assessment and stratification in the outpatient setting, measurement of high-sensitivity

cardiac troponin T (hs-cTnT) **DOES NOT MEET COVERAGE CRITERIA.**

8) For CVD risk assessment screening, evaluation, and management, homocysteine testing **DOES NOT MEET COVERAGE CRITERIA.**

9) For CVD risk assessment, measurement of novel lipid and non–lipid biomarkers (e.g., apolipoprotein AI, apolipoprotein E, B-type natriuretic peptide, cystatin C, fibrinogen, leptin, LDL subclass, HDL subclass) **DOES NOT MEET COVERAGE CRITERIA.**

10) Other than simple lipid panels (see Note 1), CVD risk panels consisting of multiple individual biomarkers intended to assess CVD **DO NOT MEET COVERAGE CRITERIA.**

11) For CVD risk assessment, measurement of serum intermediate density lipoproteins **DOES NOT MEET COVERAGE CRITERIA.**

12) For all situations, measurement of long-chain omega-3 fatty acids in red blood cell membranes, **DOES NOT MEET COVERAGE CRITERIA.**

13) All other tests for assessing CVD risk **DO NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: A simple lipid panel is generally composed of the following lipid markers:

- Total cholesterol
- HDL cholesterol
- LDL cholesterol
- Triglycerides

Certain calculated ratios, such as the total/HDL cholesterol may also be reported as part of a simple lipid panel.

Other types of lipid testing (i.e., apolipoproteins, lipid particle number or particle size, lipoprotein [a]) are not considered to be components of a simple lipid profile.

Note 2: 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk (Goff et al., 2014):
Risk factors include gender, age, race, smoking, hypertension, diabetes, total cholesterol, high- and low-density lipoprotein cholesterol. A race- and sex-specific PCE ASCVD Risk Estimator is available at: https://tools.acc.org/ldl/ascvd_risk_estimator/index.html#!/calulate/estimator/.

The 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol affirms that “the PCE is a powerful tool to predict population risk, but

it has limitations when applied to individuals." Hence a clinician-patient risk discussion can individualize risk status based on PCE, but with the inclusion of additional risk-enhancing factors. These additional factors may include:

- A family history of premature ASCVD (males, age <55 y; females, age <65 y)
- Primary hypercholesterolemia (LDL-C, 160–189 mg/dL [4.1–4.8 mmol/L); non–HDL-C 190–219 mg/dL [4.9–5.6 mmol/L])
- Metabolic syndrome (increased waist circumference, elevated triglycerides [>150 mg/dL], elevated blood pressure, elevated glucose, and low HDL-C [<40 mg/dL in men; <50 in women mg/dL] are factors; tally of 3 makes the diagnosis)
- Chronic kidney disease (eGFR 15–59 mL/min/1.73 m2 with or without albuminuria; not treated with dialysis or kidney transplantation)
- Chronic inflammatory conditions such as psoriasis, RA, or HIV/AIDS
- History of premature menopause (before age 40 y) and history of pregnancy-associated conditions that increase later ASCVD risk such as preeclampsia
- High-risk race/ethnicities (eg, South Asian ancestry)
- Lipid/biomarkers: Associated with increased ASCVD risk
- Persistently elevated, primary hypertriglyceridemia (≥175 mg/dL)
- Elevated high-sensitivity C-reactive protein (≥2.0 mg/L)
- Elevated Lp(a): A relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor especially at higher levels of Lp(a)
- Elevated apoB ≥130 mg/dL: A relative indication for its measurement would be triglyceride ≥200 mg/dL. A level ≥130 mg/dL corresponds to an LDL-C ≥160 mg/dL and constitutes a risk-enhancing factor
- ABI <0.9

III. Scientific Background

Statistics show that cardiovascular disease (including coronary heart disease, stroke and hypertension) is America's leading health problem, and the leading cause of death. According to the 2022 update of the heart disease and stroke statistics report released by the American Heart Association (AHA, 2022):

- Approximately 126.9 million people in this country suffer from some form of cardiovascular disease (encompassing coronary heart disease, heart failure, hypertension, and stroke).
- The direct and indirect costs of cardiovascular disease and stroke are about $378.0 billion and increasing every year.
- An estimated 121.5 million U.S. adults have high blood pressure, and 28.2 million have diabetes.
- Heart disease remains the number one cause of death in U.S. adults.
- On average, someone in the U.S. dies of a stroke every 3 minutes and 30 seconds.

Cardiovascular Disease Risk Assessment, continued

- Women have a higher lifetime risk of stroke than men.
- Approximately 14 percent of U.S. adults smoke cigarettes “some days” or “every day.”
- An estimated 42.4 percent of U.S. adults are obese

Cardiovascular Risk Assessment

Traditionally, the most important indicators for cardiac risk are those of a person's health history. These include factors such as family history, age, weight, exercise, and cigarette smoking status (Wilson, 2023).

Tests typically used to assess cardiovascular risk include:

1. Lipid profile or panel, which is the most important blood test for cardiac risk assessment
2. Biomarkers
3. Cardiovascular Risk Panels

Lipid Profile or Panel

A lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease and other diseases. The lipid profile typically includes measurements of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, and total cholesterol. Using these values, a laboratory may also calculate the very low-density lipoprotein (VLDL) and total cholesterol/HDL cholesterol ratio (Rosenson, 2022b).

Biomarkers

Traditional risk algorithms may miss up to 20% of cardiovascular disease (CVD) events (MacNamara et al., 2015). Numerous biomarkers have been proposed as potential risk markers for CVD. These biomarkers include but are not limited to: several apolipoproteins (A, B, AI, E, LDL, HDL), B-type natriuretic peptide, and C-reactive protein. These biomarkers have been proposed as an alternative or addition for risk stratification in CVD or as treatment targets for lipid-lowering therapy (Rosenson, 2022a; Rosenson et al., 2023; Wilson 2022). However, even the most promising biomarkers have only demonstrated modest associations and predictive ability.

Antonopoulos et al. (2022) investigated the added prognostic value of biomarkers of vascular inflammation for stable patients without known coronary heart disease on top of clinical risk factors.

The biomarkers—"C-reactive protein, interleukin-6 and tumor necrosis factor-a, arterial positron emission tomography/computed tomography and coronary computed tomography angiography–derived biomarkers of vascular inflammation, including anatomical high-risk plaque features and perivascular fat imaging”—were evaluated against the main endpoint of the difference in c-index (Δ[c-index]) with the use of inflammatory biomarkers for major adverse cardiovascular events (MACEs) and mortality, finding that

the "Biomarkers of vascular inflammation provided added prognostic value for the composite endpoint and for MACEs only (pooled estimate for Δ[c-index]% 2.9, 95% CI: 1.7-4.1 and 3.1, 95% CI: 1.8-4.5, respectively)". Coronary computed tomography angiography-related biomarkers are noteworthy as they produced the highest added prognostic benefit for MACEs, the biomarkers of which included "high-risk plaques 5.8%, 95% CI: 0.6 to 11.0, and perivascular adipose tissue (on top of coronary atherosclerosis extent and high-risk plaques): 8.2%, 95% CI: 4.0 to 12.5)". Though this may appear to be the case, the study remarked that the net clinical benefit and cost-effectiveness of using these biomarkers are still underreported and understudied (Antonopoulos et al., 2022).

Since low-grade inflammation has been linked to early development of cardiovascular disease in the young, Chiesa et al. (2022) evaluated whether circulating levels of glycoprotein acetyls (GlycA) were better able to predict the development of adverse cardiovascular disease risk profiles when compared with the more commonly used biomarker high-sensitivity CRP (C-reactive protein). Using data from a total of 3306 adolescents and young adults from the Avon Longitudinal Study of Parents and Children (mean age, 15.4 ± 0.3; n=1750) and Cardiovascular Risk in Young Finns Study (mean age, 32.1 ± 5.0; n=1556), the authors found that not only did "GlycA showed greater within-subject correlation over 9-to-10-year follow-up [for hypertension and metabolic syndrome] in both cohorts compared with CRP, particularly in the younger adolescent group (r=0.36 versus 0.07)", but GlycA was associated with multiple lifestyle-related cardiovascular disease risk factors, cardiometabolic risk factor burden, and vascular dysfunction. Moreover, in both cohorts, "only GlycA predicted future risk of both hypertension (risk ratio [RR], ≈1.1 per z-score increase for both cohorts) and metabolic syndrome (RR, ≈1.2–1.3 per z-score increase for both cohorts) in 9-to-10-year follow-up", suggesting that GlycA "may capture distinct sources of inflammation in the young and may provide a more sensitive measure than CRP for detecting early cardiovascular risk" (Chiesa et al., 2022).

Apolipoprotein B (Apo B)

Apo B is a major protein in the construction and regulation of lipids. There are two forms of apo B, apo B-48 and apo B-100. Apo B-100 is the major protein found in LDL and VLDL. Each LDL particle has one molecule of Apo B-100 per particle. Therefore, the apo B concentration may represent the amount of LDL well (Rosenson, 2022a). Increased levels of apo B have been associated with atherosclerosis development in several large-scale studies; however, apo B levels have yet to become routinely measured in clinical practice (Morita, 2016; Trompet et al., 2018).

Researchers have hypothesized that lowering apo B levels in young or middle-aged individuals will reduce the number of atherosclerosis cases later in life (Robinson et al., 2018). Further, atherosclerotic changes in retinal arteries have been associated with coronary artery disease (CAD) as well as apo B, TG, TC, and LDL-C levels (Tedeschi-Reiner et al., 2005). Lamprea-Montealegre et al. (2020) have analyzed data from 9270 participants with chronic kidney disease to determine if triglyceride-rich lipoproteins contribute to a greater CVD risk in this population; it was determined that increased apo B along with other triglyceride and cholesterol-related concentrations were associated with an increased risk for atherosclerotic CVD risk in chronic kidney disease patients. A second study (n=8570) has researched the relationship between apo B levels relative to LDL-C and non-LDL-C, as well as how these levels affect subclinical atherosclerotic cardiovascular disease (ASCVD) (Cao et al., 2019). Results showed that higher apo B levels were associated with an increase in coronary artery calcium (CAC) levels among adults older than 45 years who were not taking statins, "but provided only modest additional predictive value of apo

B for CAC prevalence, incidence, or progression beyond LDL-C or non-HDL-C (Cao et al., 2019)." An equation to predict major cardiovascular events based on apo B levels has even been developed, and when studied, this equation showed major cardiovascular event "risk prediction comparable to directly-measured apo B in high risk patients with previous coronary heart disease (Hwang, Ahn, Han, Park, & Park, 2017)."

The 2019 European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) published guidelines for the management of dyslipidaemias. These guidelines stated that "ApoB analysis is recommended for risk assessment, particularly in people with high TG [triglycerides], Diabetes mellitus (DM), obesity or metabolic syndrome, or very low LDL-C (Mach et al., 2019)." The ESC and EAS justify these recommendations by stating that the measurement of LDL-C levels in patients with dyslipidaemia may be inaccurate due to high DM or TG levels. "Because apo B provides an accurate estimate of the total concentration of atherogenic particles under all circumstances, it is the preferred measurement to further refine the estimate of ASCVD risk that is modifiable by lipid-lowering therapy (Mach et al., 2019)."

Apolipoprotein A-I (Apo-I)

Apo A-I is a lipid-binding protein which comprises HDL molecules. HDL contains two associated apolipoproteins, A-I and A-II, and together they are the primary components of the HDL molecules. Due to Apo A-I's role as a primary structural protein for HDL, it significantly factors into the density ranges of HDL, which ultimately contribute to their overall measurement (Rosenson, 2022a).

Direct measurement of apo AI has been proposed as more accurate than the traditional use of HDL level. Low levels of apo A–1 may be associated with an increased risk for CVD. Testing for apo A–1 is often performed with apolipoprotein B and reported as a ratio (apo B: apo A-1), thus providing a measure of atherogenic to antiatherogenic lipoprotein particles (Sandhu et al., 2016).

Apolipoprotein E (Apo E)

Apo E is the primary apolipoprotein found in VLDLs and chylomicrons. Apo E is essential in the metabolism of cholesterol and triglycerides and helps to clear chylomicrons and VLDL. Apo E polymorphisms have functional effects on lipoprotein metabolism. Some Apo E genotypes are more atherogenic than others, and their measurement could provide additional information of risk of coronary artery disease (Rosenson, 2022a).

B-type or Brain Natriuretic Peptide (BNP)

BNP is a hormone released by the ventricles of the heart when pressure to the cardiac muscles increases or there is volume overload. BNP is now an established biochemical marker for heart failure, as the level of BNP in plasma increases proportionally based on disease severity (Kuwahara, Nakagawa, & Nishikimi, 2018). Further, BNP has been accepted as an "independent surrogate marker of rehospitalization and death" for heart disease (Li & Wang, 2005), and exhibits both diagnostic and prognostic capabilities (Tomcsányi et al., 2018).

While BNP has shown great promise for diagnostic congestive heart failure purposes, a BNP guided heart failure treatment strategy seems to be controversial; some report that this type of treatment has

led to greater health-related costs and does not increase patient outcomes (Mark et al., 2018). Still, many drugs, such as beta blockers, amiodarone, spironolactone, and angiotensin converting enzyme inhibitors, have been beneficial in reduction of circulating BNP during the management of chronic heart failure (Li & Wang, 2005). A major limitation of BNP is that a wide range of values are observed in patients with and without heart failure; for example, obese individuals tend to have lower levels of this hormone than healthy individuals (Colucci & Chen, 2022).

Januzzi et al. (2019) used data from the GUIDing Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure (GUIDE-IT) trial to develop a greater understanding of the prognostic capabilities of amino-terminal pro-B-type natriuretic peptide (NT-proBNP) following heart failure. A total of 638 individuals participated in the study. The authors concluded that "Patients with heart failure with reduced ejection fraction whose NT-proBNP levels decreased to ≤1,000 pg/ml during GDMT [guideline-directed medical therapy] had better outcomes" (Januzzi et al., 2019). These results highlight the potential for NT-proBNP to be used as a prognostic tool following heart failure.

High Density Lipoprotein (HDL)

Apart from apolipoprotein content (AI and AII), HDL can be classified by size (small and large), by density (HDL2, HDL3), and by electrical charge (pre-beta, alpha and pre-alpha). There has been substantial interest in evaluating whether HDL subclass testing can be used to provide additional information on cardiovascular risk compared to HDL alone. HDL levels have been noted to be inversely related to CVD risk and possibly even protective against CVD. However, there are still many questions about the relationship between HDL and CVD risk, such as whether HDL levels are causative of lower CVD risk (Rosenson, 2022a; Rosenson & Durrington, 2021).

Low Density Lipoprotein (LDL)

LDL proteins are a significant risk factor in predicting atherosclerosis. The mechanism of how LDL subclass particles impact risk of CVD has not been determined although many mechanisms have been proposed. Even though LDL cholesterol levels may be normal, an elevation of small, dense LDL particles may be associated with CVD. One theory is that the small LDL particles can be more easily deposited into the intima, lead to atherosclerosis, and eventually CVD. Another is that LDL particles may upregulate the angiotensin II receptor, thereby promoting atherosclerosis (Rosenson, 2022a).

Lipoprotein(a)

Lipoprotein(a) (Lp[a]) is a low-density lipoprotein and has been determined to have atherogenic potential. Lp(a) has been proposed as an independent risk factor for coronary artery disease (CAD). Although research has shown it accumulates in atherosclerotic lesions, the actual process remains unclear. Serum levels of Lp(a) are highly determined by genetic polymorphisms, and the 90th percentile of Lp(a) levels was estimated at about 39 mg/dL. The overall degree of risk associated with Lp(a) levels appears to be modest, and the degree of risk may be mediated by other factors such as LDL levels and/or hormonal status. The standard method for measuring Lp(a) is density gradient ultracentrifugation. Although enzyme-linked immunosorbent assay (ELISA) techniques are available; they unable to distinguish between apo(a) isoforms, leading to inaccurate results (Rosenson et al., 2023). Lp(a) may have prognostic value in certain situations, such as in women with hypercholesterolemia (Grundy et al., 2019).

A study focusing on the possible role of Lp(a) in CVD was performed by Willeit et al. (2018); 26069 subjects were analyzed, and the authors found a linear relationship between elevated lp(a) levels and CVD risk at a baseline of ≥30 mg/dL and an on-statin level of ≥50 mg/dL. The baseline hazard ratios were 1.13 and 1.36 for 30-50 mg/dL and >50 mg/dL respectively, and the hazard ratios for patients on statins were 1.08 and 1.42 respectively (Willeit et al., 2018).

Mehta et al. (2020) investigated "independent and joint associations of Lp(a) and FHx [family history of coronary heart disease] with atherosclerotic cardiovascular disease (ASCVD) and CHD [coronary heart disease] among asymptomatic subjects". A total of 12149 patients were included and observed over 21 years, with Lp(a) levels measured at the first visit and remeasured at the fourth visit, nine years later, to confirm. The median age of this cohort was 54 years, and 44% of these patients had FHx. A total of 3114 ASCVD events were observed. Both FHx and elevated Lp(a) were independently associated with ASCVD, with a hazard ratio of 1.17 for FHx and a hazard ratio of 1.25 for elevated Lp(a). Patients with both FHx and elevated Lp(a) were found to have a hazard ratio of 1.43. Similar findings were found for CHD. The authors also noted that ASCVD and CHD risk reclassification and discrimination indices had improved accuracy with both FHx and Lp(a) included. The authors concluded that "elevated plasma Lp(a) and FHx have independent and additive joint associations with cardiovascular risk and may be useful concurrently for guiding primary prevention therapy decisions" (Mehta et al., 2020).

Cystatin C

Cystatin C is a protease inhibitor protein that plays a role in inflammation and obesity. Serum testing has been proposed to diagnose impaired kidney function, which in turn may be a risk factor for coronary heart disease (Rule, 2022). There is no published literature proving the effectiveness of Cystatin C as a biomarker for predicting cardiovascular risk and other confounding factors such as inflammation levels still need to be parsed out from Cystatin C. Overall, Cystatin C is not routinely used as a CVD biomarker (Sarnak, 2021).

Fibrinogen

Fibrinogen is a circulating glycoprotein that plays an important role in platelet aggregation and blood viscosity. Fibrinogen has been suggested as a possible indicator of inflammation that accompanies atherosclerosis. The independent predictive power, impact on management strategies, and clinical utility of fibrinogen measurement have shown conflicting results. One study of 150000 subjects demonstrated a log-linear relationship of fibrinogen and cardiovascular events, but another study of 90000 subjects did not find a relationship; therefore further research is required (Wilson, 2023). A recent study has reported that higher fibrinogen levels increased the risk of a stroke in large arteries or small vessels but decreased the risk of cardioembolic stroke (Maners et al., 2019).

Pieters et al. investigated the contribution of fibrinogen, as well as other biomarkers, on cardiovascular disease (CVD) mortality. A total of 4487 patients were evaluated over a period of 14 years. The authors noted that 551 patients had CVD at baseline and over the time period investigated, 321 CVD deaths occurred. Fibrinogen was found to associate ("cluster") with C-Reactive protein only and was associated with both baseline CVD and CVD mortality at follow-up. Both fibrinogen and gamma-glutamyl transferase were found to be mediators between CVD status and all-cause mortality, as well as between CVD status and CVD mortality (Pieters et al., 2020).

Leptin

Leptin is a protein secreted by fat cells and plays a role in fat metabolism. As leptin increases with obesity, it is thought to be associated with CVD. Leptin may play a role in regulating blood pressure, insulin sensitivity, inflammatory vascular responses, and more. However, a meta-analysis covering 13 studies, 4257 CVD patients, and 26710 controls indicated no significant relationship between leptin and CVD or stroke once other cardiovascular risk factors were controlled. The authors recommend further research to evaluate the effectiveness of leptin as a predictor of CVD (Wilson, 2023; Yang et al., 2017). A recent study found that, in a Chinese cohort, serum leptin levels were identified as a marker for patients with first-ever acute ischemic stroke and were also associated with stroke size and severity (Liu et al., 2019).

Drug Therapies Requiring Lipid Monitoring

Lipid-lowering Therapy with Statins

Statins, such as ezetimibe, are a type of drug often prescribed to lower lipid levels or cholesterol. Pignone (2022) has reported that statins may reduce CVD risk by 20 to 30%, regardless of initial LDL-C levels. Statins are also beneficial for the treatment of arterial stiffness, independent of their hypolipidemic effect; treatment with a high dosage of statins will decrease LDL-C levels and improve arterial stiffness levels (Reklou et al., 2020). Kongpakwattana et al. (2019) report that the use of statin therapy in combination with non-stain lipid-modifying agents is more beneficial to reduce CVD risk than using only one treatment method.

A meta-analysis of statin trials completed by Boekholdt et al. (2014) analyzed data from 38,153 patients. During the follow-up of only 5,387 patients, it was found that 6,286 major cardiac events occurred. Great variability was recorded in LDL-C, apo B and non-HDL-C levels based on fixed statin levels over a one-year period. “Among trial participants treated with high-dose statin therapy, >40% did not reach an LDL-C target <70 mg/dl,” suggesting that high-dose statin therapy effectiveness may depend on the individual (Boekholdt et al., 2014).

Antipsychotics

Several atypical antipsychotic medications, such as risperidone, sertindole and olanzapine, have been FDA approved for the treatment of psychiatric disorders, including bipolar disorder, depression, and schizophrenia; unfortunately, these medications may lead to a plethora of side effects, including dyslipidemia, hypertension, increased CVD risk, obesity, sudden cardiac death, and insulin resistance (Beauchemin et al., 2019; Polcwiartek, Kragholm, Schjerning, Graff, & Nielsen, 2016). Specifically, antipsychotic-induced corrected QT prolongation may increase the risk of Torsades de Pointes (a form of polymorphic ventricular tachycardia), leading to sudden cardiac death (Polcwiartek et al., 2016). While newer antipsychotics have been improved to lessen the pro-arrhythmic impact of their predecessors, they may contribute to cardiac death in a new way: by worsening the metabolic profile (Howell et al., 2019). It is recommended that any individuals in need of antipsychotics seriously consider the risks of these medications before accepting this type of treatment.

A ten-year study compared the CVD risk of patients with schizophrenia taking antipsychotics with healthy controls. The overall CVD risk was 5.16% in patients with schizophrenia, and 3.02% in the

healthy control group; further, risk scores were significantly higher and HDL levels were significantly lower in patients taking multiple antipsychotics (Kilicaslan, Karakilic, & Erol, 2019). A recent meta-analysis by Rotella et al. (2020) aimed to identify the long-term metabolic and cardiovascular effects of antipsychotic drugs. A total of 3013 studies were screened, and 92 were used for data analysis. The researchers have found a significantly higher risk of CVD death for sertindole users compared to risperidone users and state that "Long-term cardiovascular effects of APs [antipsychotics] deserve to be studied more extensively (Rotella et al., 2020)."

Accutane

Accutane, also known as isotretinoin, is a synthetic vitamin A derivative and oral medication often prescribed for the treatment of severe acne; it was approved by the FDA in 1982 to treat resistant, nodular acne that has not responded to conventional therapeutic measures such as systemic antibiotics (Pile & Sadiq, 2019). Unfortunately, isotretinoin therapy may cause various cardiac events, including congenital heart disease, atrial tachycardia, and cardiac remodeling (Guler et al., 2015). Akcay and Yuksel (2019) have reported that isotretinoin use may have been related to the development of Kounis syndrome (acute coronary syndrome due to a reduction of blood flow to the heart) in one patient. Alan et al. (2016) reported that isotretinoin use may have triggered premature ventricular contractions in a 33-year old woman. Karadag et al. (2012) completed a study comprised of 70 patients who were being treated with 0.5-1.0 mg/kg per day of isotretinoin; in each patient, heart rate, blood pressure, EEG, biochemical and hematologic parameters were all measured. "We found that isotretinoin did not affect P- and QT-wave measurement (Karadag et al., 2012)."

Isotretinoin may also affect serum lipid levels. Zane et al. (2006) studied 13772 patients with acne currently receiving oral isotretinoin therapy. Results showed that 31% of isotretinoin users had high cholesterol levels, 11% had high liver transaminase levels, and 44% had high triglyceride levels (Zane et al., 2006). In a more recent study, Lee et al. (2016) completed a systematic review and meta-analysis from 1960-2013 which studied the effects of oral isotretinoin use. Data was only admitted if 40 mg/day of isotretinoin was used for at least four weeks. The authors stated that "This meta-analysis showed that (1) isotretinoin is associated with a statistically significant change in the mean value of several laboratory tests (white blood cell count and hepatic and lipid panels), yet (2) the mean changes across a patient group did not meet a priori criteria for high-risk and (3) the proportion of patients with laboratory abnormalities was low (Lee et al., 2016; Zane et al., 2006)." The authors concluded by stating that these results do not support monthly laboratory testing for patients taking standard isotretinoin doses for acne purposes.

Other Cardiovascular Markers

C Reactive Protein (hsCRP)

Data from numerous studies have shown an association between elevated serum or plasma concentrations of CRP and atherosclerotic vascular disease, risk of recurrent cardiovascular events, and the incidence of initial cardiovascular events among individuals not known to have atherosclerosis (Crea, 2023).

CRP can be measured using either traditional assays or high sensitivity CRP (hs-CRP) assays. Traditional assays have limited use when screening for cardiovascular disease due to their limit of detection (3-5 mg/L). On the other hand, hs-CRP assays can detect concentrations of CRP down to 0.3 mg/L and below.

Cardiovascular Disease Risk Assessment, continued

These hs-CRP assays are used to assess cardiovascular risk because they can detect and quantify CRP within the range normally seen in asymptomatic patients (<3 mg/L). Elevated CRP levels, either alone or in combination with other cardiovascular risk factors, have been associated with a higher risk of future cardiovascular events. Studies evaluating CRP in asymptomatic populations have shown that the baseline level of CRP predicts the long-term risk of a first myocardial infarction (MI), ischemic stroke, hypertension, peripheral vascular disease, sudden cardiac death, and all-cause mortality (Crea, 2023).

Homocysteine
Homocysteine is an amino acid that is produced by the body. Elevated levels of homocysteine may result in damage to the walls of the artery, increase the potential for thrombosis and lead to advanced atherosclerosis. Hence, elevated homocysteine levels have been demonstrated to increase the risk of CVD. However, the testing of homocysteine levels is not consistently recommended because, based on current research, the lowering of plasma homocysteine levels does not necessarily lower the risk of CVD. Further research is required to support the clinical utility of lowering homocysteine levels (Rosenson, et al., 2020).

Intermediate Density Lipoproteins (IDL)
Intermediate Density Lipoproteins (remnant cholesterol or lipoproteins) are the cholesterol content of triglyceride-rich lipoproteins, which is composed of VLDL and IDL in the fasting state, and is a combination of VLDL, IDL and chylomicron remnants in the nonfasting state. It can be estimated by triglyceride (TG) levels in the absence of advanced lipoprotein testing. Elevated nonfasting plasma triglyceride is associated with increased risk for CVD (Varbo et al., 2013). Triglycerides are unlikely to directly cause CVD, thus VLDL and IDL are more commonly identified as the source of this increased risk for CVD (Jepsen et al., 2016). VLDL and IDL have been shown to be proatherogenic with both proinflammatory and prothrombotic effects (Joshi et al., 2016).

Genetic case studies have shown that elevated levels of remnant cholesterol are causally associated with both low-grade inflammation and CVD. Elevated levels of LDL cholesterol are associated with CVD, but not with low-grade inflammation. This indicates that elevated LDL cholesterol levels cause atherosclerosis without inflammation, whereas elevated remnant cholesterol levels lead to both atherosclerosis and inflammation (Varbo, Benn, & Nordestgaard, 2014; Varbo et al., 2013).

Another measure which includes IDL is Non-HDL-C, which is derived from the simple calculation of total cholesterol minus HDL-C. The Emerging Risk Factors Collaboration concluded that apoB and non-HDL-C predicted risk similar to directly measured LDL-C and that fasting did not affect the hazard ratios (HRs) (Di Angelantonio et al., 2009).

Lipoprotein-associated Phospholipase A2 (Lp-PLA2)
Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an inflammatory enzyme expressed in atherosclerotic plaques. It has been proposed that Lp– PLA2 testing may aid in detecting CVD risk due to its association with other biomarkers, such as LDL. The rationale for Lp-PLA2 as a key inflammatory biomarker is attractive because this enzyme is produced in atherosclerotic plaques with elevated expression found in CVD patients (Rosenson & Stafforini, 2012).

Numerous studies evaluate Lp-PLA2 as a predictor of cardiovascular risk (Garg et al., 2015; LPSC, 2010; Sudhir, 2006). These studies demonstrate that Lp-PLA2 is an independent predictor of CVD. Preliminary

clinical trials of Lp-PLA2 inhibitors showed some improvements in physiologic measures, such as reduction in hs-CRP (Sudhir, 2006). However, further clinical trials of Lp-PLA2 inhibitors failed to demonstrate significant improvements in patient outcomes (Mohler et al., 2008). Although Lp-PLA2 does not appear to have any predictive power with apparently healthy individuals, it may have utility for symptomatic patients. The link between the enzyme and LDL is found in the enzyme's plasma activity, which tends to vanish with treatment (R. S. Rosenson & Stafforini, 2012). De Stefano et al. (2019) stated that Lp-PLA2 may be considered as a new vascular specific biomarker to predict CVD in a population of patients with metabolic diseases.

Long-Chain Omega-3 Fatty Acids

Omega-3 fatty acids, a specific group of polyunsaturated fatty acids containing a double bond three carbons from the methyl terminus, are main building blocks of many fats and oils. Long-chain omega 3 fatty acids (≥C20, LC) include eicosapentaenoic acid (EPA, 20:5ω3), docosapentaenoic acid (DPA, 22:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) and are thought to be beneficial in the prevention of coronary heart disease (CHD) (Mozaffarian, 2019). Circulating blood levels of EPA and DHA are inversely and significantly associated with reduced CHD event risk (de Oliveira Otto et al., 2013). Blood levels of omega-3 fatty acids may be more related to CVD benefit than the daily dose of fish oil supplements (Superko et al., 2014). The blood EPA/arachidonic acid (AA) ratio may be a clinically relevant measurement as AA has atherogenic and thrombogenic metabolites. Although this ratio has substantial individual variability, an EPA/AA ratio >0.75 has been associated with a significantly lower number of major coronary events in a Japanese population (Itakura et al., 2011). Determination of blood omega-3 levels may help guide the appropriate use of dietary fish or omega-3 supplements in a personalized heart disease prevention strategy.

The relationship of fish and dietary omega-3 fatty acids and cardiovascular disease (CVD) has been investigated in numerous studies and comprehensive reviews and recommendations exist, but guidance on blood concentrations is missing. Some prospective fish oil treatment investigations report a significant reduction in CVD events, but others do not (Bosch et al., 2012; Itakura et al., 2011). A meta-analysis did not find a statistically significant relationship between omega-3 consumption and CVD mortality (Rizos, Ntzani, Bika, Kostapanos, & Elisaf, 2012). A science advisory from the AHA stated that for individuals with prevalent CHD such as a recent MI event, treatment with omega-3 PUFA supplements is reasonable; further, for patients with prevalent heart failure without preserved left ventricular function, fish oil treatment is recommended, while treatment is not recommended for patients with diabetes mellitus, prediabetes or as a method for stroke prevention (Siscovick et al., 2017).

Troponins (I, T)

Troponins are specific biomarkers for cardiac injury and are often used to diagnose myocardial infarctions. These proteins control the calcium-mediated interaction of actin and myosin in the muscle, and the cardiac versions of these proteins are unique to the heart. There are two primary categories of tests for troponins; "sensitive or contemporary" and "high-sensitive." The high-sensitive version is preferred due to its superior accuracy (Gibson, 2020; A. Jaffe, 2020; A. Jaffe, Morrow, David, 2019).

Elevated levels of troponins are proposed to predict CVD risk. Ford et al. (2016) performed a study evaluating troponin levels in 3318 men in relation to CVD risk. A hazard ratio of 2.3 for the highest quartile of troponin (≥5.2 ng/L) compared to the lowest quartile (≤3.1 ng/L) was found. The authors also

found a 5-fold reduction in coronary events when troponin levels decreased by a quarter (Ford et al., 2016).

Tang et al. (2020) evaluated the ability of high-sensitivity cardiac troponin I (hs-cTnI) to assess cardiovascular risk and mortality. A total of 5876 patients ages 66-90 years were included. A total of 1053 deaths (321 CVD-related) occurred, within a median follow-up of 6.3 years. Patients with an elevated hs-cTnI and without history of CVD had a similar mortality risk to patients with a CVD history but without an elevated hs-cTnI. However, after adjustment, elevated hs-cTnI was found to be associated with mortality risk, by a hazard ratio of 2.38 over low hs-cTnI and no CVD. Elevated hs-cTnI was found to be independently associated with incident CVD by a hazard ratio of 3.41, ASCVD (HR = 2.02) and heart failure (HR = 6.16). The authors concluded that "Hs-cTnI improves mortality and CVD risk stratification in older adults beyond traditional risk factors and improved model discrimination more than hs-cTnT for certain outcomes" (Tang et al., 2020)

Suthahar et al. (2020) "evaluate[d] associations of high-sensitivity cardiac troponin-T (cTnT) with cardiovascular disease (CVD), heart failure (HF), and mortality in community-dwelling women and men". A total of 8226 adults were included in the study. The authors detected cTnT levels in 1102 women and 2396 men. The authors found these baseline levels to be associated with a greater risk of developing CVD in women compared to men (women hazard ratio = 1.48, men hazard ratio = 1.20). Similar sex-related differences were found for heart failure and mortality. Women at 6 ng/L were also found to have significantly increased risk for CVD, HF, and mortality whereas men were only found to have significantly increased risk for CVD at the same level of cTnT (Suthahar et al., 2020).

Proprietary Testing

Cardiovascular Risk Panels/Profiles

Cardiovascular risk panels refer to combinations of cardiac markers that are used for the risk assessment of developing cardiovascular disease, major adverse cardiovascular events, or ischemic cerebrovascular events. Commercially available risk panels use different combinations of lipids, inflammatory, genetic, and metabolic markers. Risk panels report the results of multiple individual tests, whereas quantitative risk scores generally use proprietary algorithms to combine the results of multiple markers into one score. The clinical utility of risk panels is lacking as the impact of results on patient management is unknown.

Examples of commercially available cardiovascular risk panels include, but are not limited to:

1. Genova Diagnostics Cardio Check™ Panel:

    - Lipid markers (LDL; total cholesterol; HDL; triglycerides; lipoprotein (a)); total cholesterol/HDL ration; triglycerides/HDL ratio)
    - Independent risk factors (hs-CRP; homocysteine)
    - Insulin
    - Sex hormone markers (testosterone, total; sex hormone binding globulin) (Genova Diagnostics,

2023).

2. Cleveland HeartLab CVD Inflammation Testing Profile

   - F2-isoprostanes; oxidized LDL; hs-CRP; ADMA/SDMA; microalbumin; myeloperoxidase; Lp-PLA2 activity (HeartLab, 2023)

3. WellnessFX various packages:

   - Basic offerings include panels with markers such as Apo A-1 and Apo-B; the e-checkup comes with markers such as total cholesterol, HDL, LDL, and Triglycerides, the basic panel adds additional biomarkers and the "premium" panel assesses 92 markers (WellnessFX, 2023).

IV. Guidelines and Recommendations

American College of Cardiology (ACC) and the American Heart Association (AHA)

The 2019 ACC and AHA guidelines state that "Adults who are 40 to 75 years of age and are being evaluated for cardiovascular disease prevention should undergo 10-year atherosclerotic cardiovascular disease (ASCVD) risk estimation and have a clinician–patient risk discussion before starting on pharmacological therapy, such as antihypertensive therapy, a statin, or aspirin. In addition, assessing for other risk-enhancing factors can help guide decisions about preventive interventions in select individuals, as can coronary artery calcium scanning (Arnett et al., 2019)."

Laboratory testing was not addressed in this update.

The ACC and AHA published joint guidelines on the assessment of cardiovascular risk in asymptomatic patients in 2010 (Greenland et al., 2010), and updated in 2013 (Goff et al., 2014).

In adults between the ages of 20 and 79 who are free from CVD, the ACC/AHA state that it is reasonable to assess risk factors (smoking, hypertension, diabetes, total cholesterol, high density lipoprotein cholesterol) every four to six years so as to calculate 10-year CVD risk (Goff et al., 2014).

ACC/AHA also made the following recommendations on reclassification or contribution to risk assessment when high-sensitivity C-reactive protein (hs-CRP), apolipoprotein B (ApoB), glomerular filtration rate (GFR), microalbuminuria, family history, cardiorespiratory fitness, ankle-brachial index (ABI), carotid intima-media thickness (CIMT), or coronary artery calcium (CAC) score are considered in addition to the variables that are in the traditional risk scores:

1. If, after quantitative risk assessment, a risk-based treatment decision is uncertain, assessment of

one or more of the following—family history, hs-CRP, ABI or CAC may be considered to inform treatment decision making.

2. CIMT is not recommended for routine measurement in clinical practice for risk assessment for a first ASCVD event.

3. The contribution to risk assessment for a first ASCVD event using ApoB, chronic kidney disease, albuminuria, or cardiorespiratory fitness is uncertain at present (Goff et al., 2014).

The 2010 guidelines contained the following statement concerning testing for Lp-PLA2: Lipoprotein-associated phospholipase A2 might be reasonable for cardiovascular risk assessment in intermediate-risk asymptomatic adults. However, the 2013 guidelines on the assessment of cardiovascular risk do not mention Lp-PLA2 testing (Goff et al., 2014; Greenland et al., 2010).

The updated guidelines do not address arterial compliance, lipoprotein-associated phospholipase, long-chain omega-3 fatty acids, or KIF6 genotyping, endothelial function assessment as methods to assess initial CVD risk. (Goff et al., 2014; Greenland et al., 2010).

The ACC notes cutoffs of certain biomarkers for increased ASCVD risk, which are as follows: persistently elevated, primary hypertriglyceridemia ≥175 mg/dL, ≥2 mg/L hs-CRP, ≥50 mg/dL or ≥125 nmol Lp(a), ≥130 mg/dL Apo B (corresponding to >160 mg/dL LDL-C), and <0.9 ankle-brachial index (ABI) (ACC, 2018; Grundy Scott et al., 2019).

The ACC and AHA also released joint guidelines with the AAPA, ABC, ACPM, AGS, APhA, ASH, ASPC, NMA, and PCNA, stating that screening and management of dyslipidemia/hypercholesterolemia is recommended in adults with hypertension (defined as >130/80 mmHg) (Whelton et al., 2018).

2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines

This joint report discusses management of blood cholesterol. The report addresses treatments, populations of interest, and serum assessments of relevant cardiovascular biomarkers such as Apo B and lipoprotein A. The relevant recommendations are listed below:

The report notes that although measurement of Apo B may be "unreliable", persistent elevation of Apo B may be considered a risk factor. The report remarks that a level of >130 mg/dL Apo B should be considered a risk-enhancing factor [of ASCVD], as it corresponds to an LDL-C level of ≥160 mg/dL.

The report also remarks that Lp(a) is considered a risk factor for ASCVD at levels of "≥50 mg/dL or ≥125 nmol/L". However, the authors write that it should be "considered in women only in the presence of hypercholesterolemia and with the understanding that the improvement in risk prediction in adult

women in a large clinical trial was minimal".

The power of these risk factors can be seen in the "pooled cohort equation", "the single most robust tool for estimating 10-year risk in US adults 40 to 75 years of age". These algorithms have strong representative power for larger populations. However, a notable limitation of these algorithms is that they are not as accurate for individuals.

Hence a clinician-patient risk discussion can individualize risk status based on PCE, but with the inclusion of additional risk-enhancing factors. These additional factors may include:

- "A family history of premature ASCVD (males, age <55 y; females, age <65 y)
- Primary hypercholesterolemia (LDL-C, 160–189 mg/dL [4.1–4.8 mmol/L); non–HDL-C 190–219 mg/dL [4.9–5.6 mmol/L])
- Metabolic syndrome (increased waist circumference, elevated triglycerides [>150 mg/dL], elevated blood pressure, elevated glucose, and low HDL-C [<40 mg/dL in men; <50 in women mg/dL] are factors; tally of 3 makes the diagnosis)
- Chronic kidney disease (eGFR 15–59 mL/min/1.73 m2 with or without albuminuria; not treated with dialysis or kidney transplantation)
- Chronic inflammatory conditions such as psoriasis, RA, or HIV/AIDS
- History of premature menopause (before age 40 y) and history of pregnancy-associated conditions that increase later ASCVD risk such as preeclampsia
- High-risk race/ethnicities (eg, South Asian ancestry)
- Lipid/biomarkers: Associated with increased ASCVD risk
- Persistently elevated, primary hypertriglyceridemia (≥175 mg/dL)
- Elevated high-sensitivity C-reactive protein (≥2.0 mg/L)
- Elevated Lp(a): A relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor especially at higher levels of Lp(a)
- Elevated apoB ≥130 mg/dL: A relative indication for its measurement would be triglyceride ≥200 mg/dL. A level ≥130 mg/dL corresponds to an LDL-C ≥160 mg/dL and constitutes a risk-enhancing factor
- ABI < 0.9" (Grundy et al., 2019)

American Diabetes Association (ADA)

The updated ADA Standards of Medical Care in Diabetes document also includes a section focused on cardiovascular disease and risk management. Laboratory related guidelines are below:

Cardiovascular Disease Risk Assessment, continued

- "In asymptomatic patients, routine screening for coronary artery disease is not recommended as it does not improve outcomes as long as atherosclerotic cardiovascular disease risk factors are treated."
- "Consider investigations for coronary artery disease in the presence of any of the following: atypical cardiac symptoms (e.g., unexplained dyspnea, chest discomfort); signs or symptoms of associated vascular disease including carotid bruits, transient ischemic attack, stroke, claudication, or peripheral arterial disease; or electrocardiogram abnormalities."
- "In adults not taking statins or other lipid-lowering therapy, it is reasonable to obtain a lipid profile at the time of diabetes diagnosis, at an initial medical evaluation, and every 5 years thereafter if under the age of 40 years, or more frequently if indicated."
- "…risk scores and other cardiovascular biomarkers have been developed for risk stratification of secondary prevention patients (i.e., those who are already high risk because they have ASCVD) but are not yet in widespread use."
- "The American College of Cardiology/American Heart Association ASCVD risk calculator (Risk Estimator Plus) is generally a useful tool to estimate 10-year risk of a first ASCVD event…The 10-year risk of a first ASCVD event should be assessed to better stratify ASCVD risk and help guide therapy, as described below."
- "Obtain a lipid profile at initiation of statins or other lipid-lowering therapy, 4–12 weeks after initiation or a change in dose, and annually thereafter as it may help to monitor the response to therapy and inform medication adherence (ADA, 2020, 2021a)."

Also, for children and adolescents, the following recommendations were given for 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.
- 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, 2021b)."

National Lipid Association (NLA)

The NLA published a scientific statement for lipid measurements in the management of cardiovascular disease, and their recommendations (with evidence rating of "B" or higher) are included below:

- "It is recommended to follow up abnormal screening lipid measurements with fasting lipid measurement (Strength: IIa. Evidence: B-NR)
- LDL-C in adults ≥ 190 mg/dL (≥160 mg/dl in children) is recommended to be reported as possible Familial Hypercholesterolemia (Strength: I. Evidence: B-NR)
- Non-HDL-C in adults ≥ 220 mg/dL is recommended to be reported as possible inherited hyperlipidemia (Strength: I. Evidence: B-NR)
- Triglyceride concentration ≥500 mg/dL is recommended to be reported as severe hypertriglyceridemia (Strength: I. Evidence: B-NR)

Cardiovascular Disease Risk Assessment, continued

- Lipid measurements are recommended at 3-12 months for those on a stable medication regimen (Strength: I. Evidence: B-NR)
- Lipid measurements are recommended 4-12 weeks after a change in lipid treatment (Strength: I. Evidence: B-NR)
- LDL-C measurement is recommended for screening (Strength: I. Evidence: B-NR)
- LDL-C measurement is recommended on lipid therapy (Strength: I. Evidence: B-NR)
- Non-HDL-C measurement is recommended for screening (Strength: I. Evidence: B-NR)
- Non-HDL-C measurement is recommended on lipid therapy (Strength: I. Evidence: B-NR)
- Apolipoprotein B measurement may be reasonable for initial evaluation (Strength: IIb. Evidence: B-NR)
- Apolipoprotein B measurement is reasonable on lipid therapy (Strength: IIa. Evidence: B-NR)
- Apolipoprotein B measurement is recommended to facilitate diagnosis of Familial Dysbetalipoproteinemia and Familial Combined Hyperlipidemia (Strength: IIb. Evidence: B-NR)
- Lipoprotein (a) measurement is reasonable for initial evaluation in those with premature ASCVD, family history of premature ASCVD or of elevated Lp(a), history of LDL-C >190 mg/dL or suspected FH, or those with very high ASCVD risk (Strength: IIa. Evidence: B-NR)
- Lipoprotein (a) measurement may be reasonable on lipid therapy to determine those who may benefit from PCSK9 therapy who are already on maximal dose statin therapy ± ezetimibe, whose LDL-C remains above 70 mg/dl (Strength: IIb. Evidence: B-NR)"

Wilson et al. (2019) published a scientific statement to provide an update on the use of lipoprotein A [Lp(a)] in the clinical setting, particularly for atherosclerotic cardiovascular disease (ASCVD).

The Association lists the following recommendations for Lp(a) testing in clinical practice:

For adults over 20 years old, "Measurement of Lp(a) is **reasonable** to refine risk assessment for ASCVD events in:

- Individuals with a family history of first-degree relatives with premature ASCVD (<55 y[ears] of age in men, 65 y of age in women)
- Individuals with premature ASCVD (males aged <55 y and females aged <65 y), particularly in the absence of traditional risk factors
- Individuals with primary severe hypercholesterolemia (LDL ≥190 mg/dL) or suspected FH [familial hypercholesterolemia]
- Individuals at very high** risk of ASCVD to better define those who are more likely to benefit from PCSK9 inhibitor therapy."

  **Very high risk is defined as "Individuals with a history of multiple major ASCVD events or 1 major ASCVD event and multiple high-risk conditions."

The guidelines further remark that "Measurement of Lp(a) **may be reasonable** with:

- Intermediate (7.5%–19.9%) 10-y ASCVD risk when the decision to use a statin is uncertain, to improve risk stratification in primary prevention.
- Borderline (5%–7.4%) 10-y ASCVD risk when the decision to use a statin is uncertain, to improve

risk stratification in primary prevention.
- Less-than-anticipated LDL-C lowering, despite good adherence to therapy.
- A family history of elevated Lp(a).
- Calcific valvular aortic stenosis.
- Recurrent or progressive ASCVD, despite optimal lipid-lowering therapy."

Finally, 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., 2019)."

A 2021 update was published, focused on practical and analytical recommendations (Wilson et al., 2021):

"Highlights include the following:

1. It is acceptable to screen with nonfasting lipids.
2. Non-high-density lipoprotein HDL-cholesterol (non-HDL-C) is measured reliably in either the fasting or the nonfasting state and can effectively guide ASCVD prevention.
3. Low density lipoprotein cholesterol (LDL-C) can be estimated from total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) measurements. For patients with LDL-C>100 mg/dL and TG ≤150 mg/dL it is reasonable to use the Friedewald formula. However, for those with TG 150-400 mg/dL the Friedewald formula for LDL-C estimation is less accurate. The Martin/Hopkins method is recommended for LDL-C estimation throughout the range of LDL-C levels and up to TG levels of 399 mg/dL. For TG levels ≥400 mg/dL LDL-C estimating equations are currently not recommended and newer methods are being evaluated.
4. When LDL-C or TG screening results are abnormal the clinician should consider obtaining fasting lipids.
5. Advanced lipoprotein tests using apolipoprotein B (apoB), LDL Particle Number (LDL-P) or remnant cholesterol may help to guide therapeutic decisions in select patients, but data are limited for patients already on lipid lowering therapy with low LDL-C levels. Better harmonization of advanced lipid measurement methods is needed. Lipid measurements are recommended 4-12 weeks after a change in lipid treatment. Lipid laboratory reports should denote desirable values and specifically identify extremely elevated LDL-C levels (≥190 mg/dL at any age or ≥160 mg/dL in children) as severe hypercholesterolemia. Potentially actionable abnormal lipid test results, including fasting triglycerides (TG) ≥500 mg/dL, should be reported as hypertriglyceridemia. Appropriate use and reporting of lipid tests should improve their utility in the management of persons at high risk for ASCVD events."

Centers for Disease Control and Prevention (CDC)

The CDC highlights the importance of cardiovascular disease biomarkers and has developed a reference

laboratory and clinical standardization program to provide reference measurements for HDL-C, LDL-C, TG and total cholesterol (TC). The accuracy of the labs that analyze these biomarkers is also monitored by the CDC (CDC, 2023a).

The CDC notes that several health conditions increase the risk of heart disease including smoking, diabetes mellitus, obesity, high blood pressure, excessive alcohol use, physical inactivity, and unhealthy blood cholesterol levels. It is stated that "High blood cholesterol usually has no signs or symptoms. The only way to know whether you have high cholesterol is to get your cholesterol checked. Your health care team can do a simple blood test, called a "lipid profile," to measure your cholesterol levels (CDC, 2023b)."

The CDC has also developed the Lipids Standardization Program (LSP). This program ensures that the measurements reported in research studies and clinical laboratories are accurate. Blinded samples traceable to the CDC Reference Laboratory are provided to participants. The samples will be measured for total cholesterol (TC), glycerides (TG), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-I (apo A-I), and apolipoprotein B (apo B). LSP participants report their results from the provided samples back to the CDC where these results are then analyzed; if results are accurate, those laboratories receive a certificate and are considered CDC-certified (CDC, 2017b).

Centers for Disease Control and Prevention (CDC)/American Heart Association (AHA)

In 2002, the CDC and AHA held the "CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Applications to Clinical and Public Health Practice" and released these recommendations in 2003. In this workshop, they looked at evidence surrounding multiple inflammatory markers and based on all considerations, they stated that "it is most reasonable to limit current assays of inflammatory markers to hs-CRP, measured twice, either fasting or nonfasting, with the average expressed in mg/L, in metabolically stable patients." More specifically, they indicate that the two measurements of hs-CRP should optimally occur two weeks apart. If results are abnormal, hs-CRP testing should be repeated and the patient should be examined for sources of infection or inflammation. Furthermore, they provide the following recommendation specific to hs-CRP: "On the basis of the available evidence, the Writing Group recommends against screening of the entire adult population for hs-CRP as a public health measure. The Writing Group does conclude that it is reasonable to measure hs-CRP as an adjunct to the major risk factors to further assess absolute risk for coronary disease primary prevention. At the discretion of the physician, the measurement is considered optional, based on the moderate level of evidence (Evidence Level C). In this role, hs-CRP measurement appears to be best employed to detect enhanced absolute risk in persons in whom multiple risk factor scoring projects a 10-year CHD risk in the range of 10% to 20% (Evidence Level B). However, the benefits of this strategy or any treatment based on this strategy remain uncertain. The finding of a high relative risk level of hs-CRP (>3.0 mg/L) may allow for intensification of medical therapy to further reduce risk and to motivate some patients to improve their lifestyle or comply with medications prescribed to reduce their risk. Individuals at low risk (<10% per 10 years) will be unlikely to have a high risk (>20%) identified through hs-CRP testing. Individuals at high risk (>20% risk over 10

years) or with established atherosclerotic disease generally should be treated intensively regardless of their hs-CRP levels, so the utility of hs-CRP in secondary prevention appears to be more limited" (Pearson et al., 2003).

American Heart Association (AHA)

In 2022, the AHA published a scientific statement to highlight the history, biology, pathophysiology, and emerging clinical evidence in the lipoprotein a (Lp[a]) field, where they addressed the "key knowledge gaps and future directions required to mitigate the atherosclerotic cardiovascular disease risk attributable to elevated Lp(a) levels." In this statement, they noted that "levels of Lp(a) have not been shown to substantially change across the life course, although some variability occurs, as documented by intraindividual temporal variability in serial measurements from placebo-treated subjects in clinical trials." They also note that "various organizations have proposed to obtain a level once in every adult" (Reyes-Soffer et al., 2022).

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:

- Screening guidelines for dyslipidemia vary by age group;
- Although ASCVD risk in young adults is low, adults older than 20 years should be evaluated for dyslipidemia every 5 years as part of a global risk assessment
- Middle-aged individuals (Men 45-65 years, Women 55-65 years) should be screened for dyslipidemia at least every 1 to 2 years.
- All individuals with diabetes should be screened with a lipid profile at the time of diagnosis and annually thereafter. Some individuals with diabetes can be screened less frequently based on clinical considerations
- Annual screen for dyslipidemia for adults over 65 is recommended
- 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
- 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
- Direct measurement of LDL-C should be used to assess LDL-C in certain high-risk individuals, such as those with fasting TG concentrations greater than 250 mg/dL or those with diabetes or known vascular disease
- Apolipoproteins, Apo B and/or an apo B/apo A1 ratio calculation and evaluation may be useful in at-risk individuals.
- hsCRP is recommended to stratify ASCVD risk in individuals with a standard risk assessment that is

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borderline, or in those with an intermediate or higher risk with an LDL-C concentration <130 mg/dL.

- Lp-PLA2 measurement, is recommended when it is necessary to further stratify an individual's ASCVD risk, especially in the presence of hsCRP elevations.
- The routine measurement of homocysteine, uric acid, plasminogen activator inhibitor-1, or other inflammatory markers is not recommended because the benefit of doing so is not sufficiently proven.
- Coronary artery calcification (CAC) measurement has been shown to be of high predictive value and is useful in refining risk stratification
- Carotid intima media thickness (CIMT) may be considered to refine risk stratification (Jellinger et al., 2017).

The AACE/ACE published an updated algorithm in 2020. This algorithm focuses on "management of dyslipidemia and prevention of cardiovascular disease" and "complements" the above guidelines but includes information not available in 2017. Their relevant recommendations are listed below:

The guideline lists Apo B, LDL, Lp(a), and hs-CRP as biomarkers that may be "considered" in assessment of ASCVD risk for patients. The guideline also remarks that "measurement of apo B is useful in assessing the success of lipid-lowering therapy, since apo B may remain above goal after achieving the LDL-C goal." Apo B is listed as a component of treatment goals, alongside LDL-C, Non-HDL-C, and TG [triglycerides].

The guideline recommends "considering" measurement of Lp(a) (lipoprotein A) in the following settings:

- "All patients with clinical ASCVD, especially premature or recurrent ASCVD despite LDL-C lowering;
- Individuals with a family history of premature ASCVD and/or increased Lp(a);
- Individuals with South Asian or African ancestry, especially with a family history of ASCVD or increased Lp(a);
- Individuals with a 10-year ASCVD risk ≥10% (primary prevention setting), in order to stratify risk;
- Patients with a personal or family history of aortic valve stenosis;
- Patients with refractory elevations of LDL-C despite aggressive LDL-C-lowering therapy (i.e., statin resistance)" (AACE, 2021).

The AACE also published a "consensus statement" on the "comprehensive type 2 diabetes management algorithm". The guideline includes a set of PowerPoint slides at the bottom, which recommend measuring Lp(a) in the following settings: presence of family history of premature ASCVD and/or increased Lp(a), and all patients with premature or recurrent ASCVD despite LDL-C lowering (Garber et al., 2020).

European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Guidelines for the Management of Dyslipidaemias

The ESC published 2021 guidelines on cardiovascular disease prevention in clinical practice. Their

Cardiovascular Disease Risk Assessment, continued

recommendations for CVD risk assessment are included below (Visseren et al, 2021):

New or revised	Recommendations in 2013 version	Class	Recommendations in 2021 version	Class
Risk factors and clinical conditions – section 3				
New			In apparently healthy people <70 years of age without established ASCVD, DM, CKD, genetic/rarer lipid or BP disorders, estimation of 10-year fatal and nonfatal CVD risk with SCORE2 is recommended.	I
New			In apparently healthy people ≥70 years of age without established ASCVD, DM, CKD, genetic/rarer lipid or BP disorder, estimation of 10-year fatal and nonfatal CVD risk with SCORE2-OP is recommended.	I
New			Patients with established ASCVD and/or DM and/or moderate-to-severe renal disease and/or genetic/rarer lipid or BP disorders are to be considered at high or very high CVD risk.	I
New			A stepwise treatment-intensification approach aiming at intensive risk factor treatment is recommended for apparently healthy people at high or very high ASCVD risk, as well as patients with established ASCVD and/or DM, with consideration of CVD risk, treatment benefit of risk factors, risk modifiers, comorbidities, and patient preferences.	I
New			Treatment of ASCVD risk factors is recommended in apparently healthy people without DM, CKD, genetic/rarer lipid or BP disorders who are at very high CVD risk (SCORE2 ≥7.5% for age under 50; SCORE2 ≥10% for age 50–69; SCORE2-OP ≥15% for age ≥70).	I
New			An informed discussion about CVD risk and treatment benefits tailored to the needs of a patient is recommended.	I
New			It is recommended that mental disorders with either significant functional impairment or decreased use of healthcare systems be considered as influencing	I
New			Treatment of ASCVD risk factors should be considered in apparently healthy people without DM, CKD, genetic/rarer lipid, or BP disorders who are at high CVD risk (SCORE2 2.5 to <7.5% for age under 50; SCORE2 5 to <10% for age 50–69; SCORE2-OP 7.5 to <15% for age ≥70 years), taking ASCVD risk modifiers, lifetime risk and treatment benefit, and patient preferences into account.	IIa
New			In apparently healthy people, after estimation of 10-year fatal and non-fatal CVD risk, lifetime risk and treatment benefit, risk modifiers, frailty, polypharmacy, and patient preferences should be considered.	IIa
New			Presence of migraine with aura should be considered in CVD risk assessment.	IIa
New			Assessment of CVD risk should be considered in men with ED.	IIa
New			In women with a history of premature or stillbirth, periodic screening for hypertension and DM may be considered.	IIb
New			Assessment of total CVD risk may be considered in adults with chronic inflammatory conditions.	IIb
New			Avoidance of combined hormonal contraceptives may be considered in women with migraine with aura.	IIb

Cardiovascular Disease Risk Assessment, continued

New or revised	Recommendations in 2013 version	Class	Recommendations in 2021 version	Class
Risk factors and clinical conditions – section 3				
New			In apparently healthy people <70 years of age without established ASCVD, DM, CKD, genetic/rarer lipid or BP disorders, estimation of 10-year fatal and nonfatal CVD risk with SCORE2 is recommended.	I
New			In apparently healthy people ≥70 years of age without established ASCVD, DM, CKD, genetic/rarer lipid or BP disorder, estimation of 10-year fatal and nonfatal CVD risk with SCORE2-OP is recommended.	I
New			Patients with established ASCVD and/or DM and/or moderate-to-severe renal disease and/or genetic/rarer lipid or BP disorders are to be considered at high or very high CVD risk.	I
New			A stepwise treatment-intensification approach aiming at intensive risk factor treatment is recommended for apparently healthy people at high or very high ASCVD risk, as well as patients with established ASCVD and/or DM, with consideration of CVD risk, treatment benefit of risk factors, risk modifiers, comorbidities, and patient preferences.	I
New			Treatment of ASCVD risk factors is recommended in apparently healthy people without DM, CKD, genetic/rarer lipid or BP disorders who are at very high CVD risk (SCORE2 ≥7.5% for age under 50; SCORE2 ≥10% for age 50–69; SCORE2-OP ≥15% for age ≥70).	I
New			An informed discussion about CVD risk and treatment benefits tailored to the needs of a patient is recommended.	I
New			It is recommended that mental disorders with either significant functional impairment or decreased use of healthcare systems be considered as influencing	I
New			Treatment of ASCVD risk factors should be considered in apparently healthy people without DM, CKD, genetic/rarer lipid, or BP disorders who are at high CVD risk (SCORE2 2.5 to <7.5% for age under 50; SCORE2 5 to <10% for age 50–69; SCORE2-OP 7.5 to <15% for age ≥70 years), taking ASCVD risk modifiers, lifetime risk and treatment benefit, and patient preferences into account.	IIa
New			In apparently healthy people, after estimation of 10-year fatal and non-fatal CVD risk, lifetime risk and treatment benefit, risk modifiers, frailty, polypharmacy, and patient preferences should be considered.	IIa
New			Presence of migraine with aura should be considered in CVD risk assessment.	IIa
New			Assessment of CVD risk should be considered in men with ED.	IIa
New			In women with a history of premature or stillbirth, periodic screening for hypertension and DM may be considered.	IIb
New			Assessment of total CVD risk may be considered in adults with chronic inflammatory conditions.	IIb
New			Avoidance of combined hormonal contraceptives may be considered in women with migraine with aura.	IIb

The authors include additional recommendations for CVD risk estimation and modification that are tabulated in the full guideline.

Cardiovascular Disease Risk Assessment, continued

2019 guidelines from the ESC and EAS provided the following recommendations:

- "Lp(a) measurement should be considered at least once in each adult person's lifetime to identify those with very high inherited Lp(a) levels >180 mg/dL (>430 nmol/L) who may have a lifetime risk of ASCVD equivalent to the risk associated with heterozygous familial hypercholesterolemia.
- "Persons with documented ASCVD, type 1 or type 2 DM (T1DM and T2DM, respectively), very high levels of individual risk factors, or chronic kidney disease (CKD) are generally at very-high or high total CV risk. No risk estimation models are needed for such persons…"
- ApoB analysis is recommended for risk assessment, particularly in people with high TG, DM, obesity or metabolic syndrome, or very low LDL-C. It can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis, and management, and may be preferred over non-HDL-C in people with high TG, DM, obesity, or very low LDL-C.
- CAC score assessment with CT may be helpful in reaching decisions about treatment in people who are at moderate risk of ASCVD. Obtaining such a score may assist in discussions about treatment strategies in patients where the LDL-C goal is not achieved with lifestyle intervention alone and there is a question of whether to institute LDL-C-lowering treatment. Assessment of arterial (carotid or femoral) plaque burden on ultrasonography may also be informative in these circumstances (Mach et al., 2019)."

Total cholesterol may be used to estimate total cardiovascular risk. LDL-C is recommended to be used as the primary lipid analysis for diagnosis, management, screening, and risk estimation. HDL-C and Non-HDL-C are also strong, independent risk factors (Catapano et al., 2016).

Apo B, Lp(a), Apo B/Apo A-I, and Non-HDL-C/HDL-C may all be used as alternative markers for cardiovascular risk. The guidelines note that measuring Apo B and Apo A-I is convenient, accurate, does not require fasting, and is not susceptible to TG levels. The guidelines also recommend against routine measurement of Apo C-III as its use is unknown (Catapano et al., 2016).

In the 2021 ESC Guidelines on cardiovascular disease prevention, the authors stated that "New studies confirm that C-reactive protein has limited additional value. There is renewed interest in lipoprotein(a), but it too provides limited additional value in terms of reclassification potential. Cardiac biomarkers are promising, but further work is needed" (Visseren et al., 2021).

European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD)

This joint guideline was published for "diabetes, pre-diabetes, and cardiovascular diseases". Their relevant recommendations are listed below:

- Routine assessment of novel biomarkers is not recommended for CV risk stratification.

The guideline noted that "the addition of circulating biomarkers for CV risk assessment has limited clinical value" and stated that "in patients with DM [diabetes mellitus] without known CVD,

Cardiovascular Disease Risk Assessment, continued

measurement of C-reactive protein or fibrinogen (inflammatory markers) provides minor incremental value to current risk assessment". Cosentino et al. (2020) also noted high-sensitivity cardiac troponin T as not adding incremental "discriminative power" for patients with DM without known CVD (Cosentino et al., 2020), although elevated high-sensitivity cardiac troponin T was noted as an independent predictor of renal decline and CV events in patients with type 1 diabetes (Cosentino et al., 2020)

Endocrine Society (ES)

This guideline was published with the intent to assess and treat dyslipidemia in patients with endocrine disorders. Their relevant recommendations are listed below:

- "In adults with endocrine disorders, we recommend a lipid panel for the assessment of triglyceride levels and for calculating low-density lipoprotein cholesterol."

- "In adults with endocrine disorders, we recommend conducting a cardiovascular risk assessment by evaluating traditional risk factors, including the calculation of 10-year atherosclerotic cardiovascular disease risk using a tool such as the Pooled Cohort Equations."

    - "In adults with endocrine disorders at borderline or intermediate risk (10-year atherosclerotic cardiovascular disease risk 5%–19.9%), particularly those with additional risk-enhancing factors, in whom the decision about statin treatment and/or other preventive interventions is uncertain, we suggest measuring coronary artery calcium to inform shared decision making."

    - "In adults with endocrine disorders at borderline or intermediate risk (10-year atherosclerotic cardiovascular disease risk 5%–19.9%), particularly those with additional risk-enhancing factors, in whom the decision about statin treatment and/or other preventive interventions is uncertain, we suggest measuring coronary artery calcium to inform shared decision making."

- "In adults with endocrine disorders at borderline or intermediate risk (10-year atherosclerotic cardiovascular disease risk 5%–19.9%), particularly those with additional risk-enhancing factors, in whom the decision about statin treatment and/or other preventive interventions is uncertain, we suggest measuring coronary artery calcium to inform shared decision making."

The guideline also remarks that certain "advanced" lipid testing (assessment of markers such as Apo B, lipid fractionation, and Lp(a)) may be helpful in "characterizing" lipid abnormalities, but "add little" to risk prediction beyond the standard lipid profile.

The guideline goes on to discuss Lp(a), noting that the marker can be helpful in assessing familial risk,

but adds "little" in terms of global risk assessment across the general population. The guideline acknowledges other evidence supporting Lp(a)'s use as a marker to manage treatment. Other serum biomarkers and biomarker panels were also considered to add "little" to global risk assessment. Finally, the guideline recommends the use of hs-CRP as a "risk-enhancing factor that may drive more aggressive treatment or the need for advanced risk assessment" (Newman et al., 2020).

Rosenzweig et al. (2019) published on the Primary Prevention of ASCVD and T2DM in Patients at Metabolic Risk. A summary of the recommendations is included below (Rosenzweig et al., 2019):

- "In individuals aged 40–75 years in the office setting, we suggest providers screen for all five components of metabolic risk at the clinical visit. The finding of at least three components should specifically alert the clinician to a patient at metabolic risk (at higher risk for atherosclerotic cardiovascular disease and type 2 diabetes mellitus).
- In individuals aged 40-75 years in the office setting who do not yet have atherosclerotic cardiovascular disease or type 2 diabetes mellitus and already have at least one risk factor, we advise screening every 3 years for all five components of metabolic risk as part of the routine clinical examination.
- To establish metabolic risk in the general population, we recommend that clinicians measure waist circumference as a routine part of the clinical examination.
- In individuals previously diagnosed with prediabetes, we suggest testing at least annually for the presence of overt type 2 diabetes mellitus.
- We recommend that all individuals at metabolic risk in the office setting have their blood pressure measured annually and, if elevated, at each subsequent visit.
- For individuals with elevated blood pressure above 130 mmHg systolic and/or 80 mmHg diastolic who are not documented as having a history of hypertension, we recommend confirmation of elevated blood pressure on a separate day within a few weeks or with a home blood pressure monitor."

Components of "metabolic risk" are defined as:

- elevated blood pressure
- increased waist circumference
- elevated fasting triglycerides
- low high-density lipoprotein-cholesterol, and
- elevated glycemia.

American Society for Clinical Pathology

The ASCP recommends against routinely ordering expanded lipid panels (such as particle sizing or nuclear magnetic resonance) as screening for cardiovascular disease (ASCP, 2016).

Cardiovascular Disease Risk Assessment, continued

In 2022, the ASCP published a set of clinical recommendations aiming to provide the guidance and the tools for assessment of ASCVD risk with the goal of appropriately targeting treatment approaches for prevention of ASCVD events, as shown below:

"1. Assessing a patient's risk for ASCVD is the foundation of preventive cardiology and the initial step for determining the appropriateness and intensity of preventive treatment.
2. In primary prevention, global risk scoring is the initial stage for ASCVD risk assessment, providing a calculation of ASCVD risk from a set of standard office-based risk factors for a specified duration (e.g., 10 years) of time, from which a clinician-patient risk discussion is used to discuss the best ways to reduce CVD risk.
3. The presence, quantity, and/or extent of one or more risk enhancing factors, including premature family history, persistently elevated LDL-C, or CKD, as well as severity of certain inflammatory factors such as hsCRP and laboratory measures such as lp(a), can further inform the treatment decision.
4. In women, it is important to take a comprehensive reproductive history from menarche to menopause, including preeclampsia, premature menopause, and autoimmune disease as "risk-enhancing" factors.
5. Race/ethnicity may have a significant impact on the validity of current risk assessment tools and certain higher risk race/ethnic groups may further inform the use of preventive therapy.
6. Social determinants of health may exert independent effects beyond race/ethnicity and need also to be part of the clinician-patient discussion when discussing the most appropriate ways to optimize ASCVD risk.
7. Among subclinical atherosclerotic disease screening tests, CAC is probably the most useful, providing substantial improvement of risk reclassification over global risk scoring in most primary prevention groups, including diabetes. In addition to the consideration of risk enhancing factors (discussed earlier), CAC testing can be used to further inform treatment decisions for preventive therapy, including statin and aspirin use in particular.
8. The use of ABI for assessment of PAD is also valuable and can improve risk reclassification beyond global risk scoring.
9. Carotid ultrasound imaging, if accompanied by carotid plaque assessment may also be useful for risk assessment, especially as an option when CAC scoring is not available.
10. In patients with pre-existing ASCVD, stratification into those at highest risk (e.g., very high risk ASCVD status) for more aggressive treatment is based on the history of multiple major ASCVD events or one major event and multiple high-risk conditions. Moreover, those with recurrent ASCVD events in the short-term define an extreme risk condition warranting even more aggressive risk factor management." (Wong et al., 2022)

National Institute for Health and Care Excellence (NICE)

A baseline lipid profile should be taken before treatment. This should include total cholesterol, HDL cholesterol, non-HDL, and triglyceride levels. Total and HDL cholesterol should be measured for best estimate of CVD risk.

Omega-3 compounds have “no evidence” to help prevent CVD and NICE recommends against distribution of these compounds for CVD treatment (NICE, 2016).

US Preventive Services Task Force (USPSTF)

The USPSTF Task Force Recommendations include periodic assessment of cardiovascular risk factors from ages 40 to 75 years, including measurement of total cholesterol, LDL-C, and HDL-C levels. The optimal intervals for cardiovascular risk assessment are uncertain. Based on other guidelines and expert opinion, reasonable options include annual assessment of blood pressure and smoking status and measurement of lipid levels every 5 years. Shorter intervals may be useful for persons at higher risk, and longer intervals are appropriate for persons who are regularly at average risk (Bibbins-Domingo et al., 2017).

The USPSTF found insufficient evidence that screening for dyslipidemia in younger adults influences cardiovascular outcomes, and no studies that evaluated the effects of screening vs no screening, treatment vs no treatment, or delayed vs earlier treatment in adults in this age group. Thus, the USPSTF recommends neither for nor against screening for dyslipidemia in this age group. The USPSTF also noted there was insufficient evidence to assess the balance of benefits and harms of screening for dyslipidemia in children and adolescents (Chou et al., 2016).

The USPSTF states that “current evidence is insufficient to assess the benefits and harms of adding ankle-brachial index (ABI), high-sensitivity C-reactive protein (hsCRP) level, or coronary artery calcium (CAC) score to traditional risk assessment for cardiovascular disease (CVD) in asymptomatic adults to prevent CVD events (USPSTF, 2018).” However, the USPSTF recommends screening for abnormal blood glucose for adults aged 40-70 who are overweight or obese (USPSTF, 2015).

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, 2020). In adults, however, The USPSTF recommends screening for hypertension (persons 18 years or older) with office blood pressure measurement (OBPM). The USPSTF recommends obtaining blood pressure measurements outside of the clinical setting for diagnostic confirmation before starting treatment (USPSTF, 2021).

Finally, screening for obesity in children 6 years or older is recommended (Bibbins-Domingo et al., 2017).

Canadian Cardiovascular Society (CCS)

The CCS published updated recommendations on the management of dyslipidemia for the prevention of cardiovascular disease in adults. A summary of the society’s recommendations that are relevant to CVD risk assessment is provided below:

Cardiovascular Disease Risk Assessment, continued

- "Among women whose pregnancy was complicated by the hypertensive disorders of pregnancy -- gestational hypertension and/or preeclampsia -- or a preterm birth before 34 weeks' gestation, a stillbirth and/or a placental abruption, we recommend screening with a comprehensive lipid panel at least 12 weeks postpartum. These women have a higher risk of premature CVD and stroke within 10-15 years after the affected pregnancy (Strong Recommendation, Moderate Quality Evidence).

- To assist with decisions about initiating lipid-lowering pharmacotherapy in a nonpregnant woman who had one or more of these pregnancy complications, we recommend referral to a specialized postpartum cardiovascular health clinic or specialized lipid clinic, if locally available. If such resources are not locally available, we recommend using standard risk assessment tools to decide about lipid-lowering pharmacotherapy. However, when interpreting their 10-year CVD risk using a risk calculator, it is important to note that most women in this group will be found to have a low calculated absolute risk of CVD, short-term, which may give a false sense of reassurance to both the patient and her health care provider. (Weak Recommendation; Low-Quality Evidence).

- For any patient with triglycerides > 1.5mM, use non-HDL-C or apoB instead of LDL-C as the preferred lipid parameter for initial screening and treatment target (< 2.6 mM for non-HDL-C or < 0.8 g/L for apoB) in intermediate or high risk individuals (Strong Recommendation, High-Quality Evidence).

- We recommend measuring lipoprotein (a) level once in a person's lifetime as a part of the initial lipid screening (Strong Recommendation; High-Quality Evidence).

- We suggest that CAC [coronary artery calcium] screening using computed tomography imaging may be considered for asymptomatic adults 40 years or older and at intermediate risk (FRS 10%-20%) for whom treatment decisions are uncertain (Weak Recommendation, Moderate-Quality Evidence).

- We recommend that CAC screening using computed tomography imaging not be undertaken for: (1) high-risk individuals; (2) patients receiving statin treatment; or (3) most asymptomatic, low-risk adults (Strong Recommendation; Moderate-Quality Evidence).

- We suggest that CAC screening may be considered for a subset of low-risk individuals 40 years or older." (Weak Recommendation; Low-Quality Evidence)." (Pearson et al., 2021)

The 2021 guidelines affirmed those from 2016, stating that "Screening should be repeated every 5 years for men and women aged 40-75 years using the modified FRS or Cardiovascular Life Expectancy Model (CLEM) to guide therapy to reduce major CV events."

A revision of the 2016 recommendation is the role of Lp(a): "Lp(a) is not currently considered a treatment target and repeat measures are therefore not indicated." Moreover, non-fasting lipid testing is recommended during the CV risk assessment, and "It is now generally preferable to follow non-HDL-C or

ApoB levels over LDL-C when interpreting lipid results, particularly when TG are ≥ 1.5 mmol/L" (Pearson et al., 2021).

HIV Medicine Association of the Infectious Diseases Society of America

HIV-infected patients commonly develop dyslipidemia after starting antiretroviral therapy (ART). The lipid abnormalities developed in HIV-infected patients are associated with increased cardiovascular risk. HIV Medicine Association of the Infectious Diseases Society of America have updated their guidelines in 2013 to include a new section on metabolic comorbidities. They recommend obtaining a fasting lipid profile prior to and within 1-3 months after starting ART and every 6-12 months in all patients. The 2020 update affirms this, as under the workup for routine healthcare maintenance considerations for persons with HIV, they recommend the following steps: "Lipid profile: perform every 5 years if normal; more frequently if abnormal or other cardiovascular risk factors present (every 6–12 months); if abnormal, repeat fasting", and asks that clinicians "Follow the atherosclerotic cardiovascular disease risk calculator. Consider testing 1–3 months after starting or changing ART" (Thompson et al., 2020).

The Association also notes that HbA1c may be tested or used for screening and states that a lower cutoff of 5.8% for diabetes may be used for patients on ART instead of the higher 6.5%. Finally, the Association recommends measuring HbA1c every six months in patients with diabetes (Aberg et al., 2014; Thompson et al., 2020).

According to the Association, an initial evaluation and an immediate follow-up for persons with HIV includes "A comprehensive present and past medical history that includes HIV-related information, medication/social/family history…, review of systems, and physical examination… should be obtained for all patients upon initiation of care, ideally at the first visit or, if not feasible, as soon as possible thereafter", and this includes testing for "Cardiovascular disease and risk factors, including hyperlipidemia, hypertension, diabetes mellitus, smoking" (Thompson et al., 2020).

U.S. Department of Veterans Affairs (VA) and U.S. Department of Defense (DoD)

The United States VA and DoD published a joint guideline regarding management of dyslipidemia for reducing CVD risk in adults. Their relevant recommendations are listed below:

- "For primary prevention in patients over age 40 and not on statin therapy who have not developed new cardiovascular risk factors (e.g., diabetes, hypertension, tobacco use), we suggest against offering a cardiovascular disease risk assessment more frequently than every five years."
- "For primary prevention in patients not on statin therapy, we suggest against routinely ordering a lipid panel more frequently than every 10 years."
- "For cardiovascular risk assessment in primary prevention, we suggest using a 10-year risk calculator."
- "We suggest against the routine use of coronary artery calcium testing."

Cardiovascular Disease Risk Assessment, continued

- "We suggest against the routine use of additional risk markers (e.g., high-sensitivity C-reactive protein, ankle-brachial index, coronary artery calcium) when assessing cardiovascular risk."

The guideline also remarks that several other markers, such as "coronary artery calcium (CAC), high-sensitivity C-reactive protein, ankle–brachial index, and apolipoprotein evaluations" have been proposed as useful tools to determine risk. However, these markers have been deemed "limited in further refining risk". Although CAC was considered to best of the markers listed, the guideline still recommended against routine CAC testing.

The guideline also recommends against "routine lipid level testing for risk assessment and monitoring, unless it is specifically intended to guide decision making" (O'Malley et al., 2020).

2021 European Society of Cardiology (ESC) and Other Societies on Cardiovascular Disease Prevention in Clinical Practice

In 2021, the Seventh Joint Task Force of the ESC and Other Societies on Cardiovascular Disease Prevention in Clinical Practice published guidelines on cardiovascular disease prevention in clinical practice.

The 2021 update has the same goals and targets for LDL-C, BP, and glycemic control in patients with diabetes mellitus as the previous 2016 guideline.

The authors state that routine assessment of circulating or urinary biomarkers is still not recommended for CVD risk stratification. The Task Force states that there is conflicting data on the utility of these biomarkers (such as hsCRP, various apolipoproteins, etc.) and that new studies confirm that C-reactive protein has "limited additional value." The authors concede that there is renewed interest in lipoprotein a, but that it doesn't add additional value in risk stratification. While cardiac biomarkers show promise, additional clinical studies are necessary.

The Task Force recommends repeating risk assessment every 5 years, and more often for higher risk patients. However, the Task Force only recommends this screening procedure for men >40 years and women >50 years, declaring that, though it is not cost-effective, a systematic CVD risk assessment is shown to "increase detection of CV risk factors" (Visseren et al., 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

Cardiovascular Disease Risk Assessment, continued

80061	Lipid panel
82172	Apoliporotein
82465	Total serum cholesterol
82610	Cystatin C
83090	Homocysteine
83695	Lipoprorotein (a)
83698	Lipoprotein-associated phospholipase A2 (Lp-PLA2)
83700	Lipoprotein, blood; electorophoretic separation and quantitation
83701	Lipoprotein, blood; high resolution fractionation and quantitation of lipoproteins including lipoprotein subclasses when performed (e.g., electrophoresis, ultracentrifugation) [VAP cholesterol test]
83704	Lipoprotein, blood; quantitation of lipoprotein particle numbers and lipoprotein particle subclasses (e.g., by nuclear magnetic resonance spectroscopy)
83718	Lipoprotein, direct measurement; high density cholesterol (HDL cholesterol)
83719	Lipoprotein, direct measurement; VLDL cholesterol
83721	Lipoprotein, direct measurement; low density cholesterol (LDL cholesterol)
83722	Lipoprotein, direct measurement; small dense LDL cholesterol
83880	Natriuretic peptide
84478	Triglycerides
84484	Troponin, quantitative
85384	Fibrinogen; activity
85415	Fibrinolytic factors and inhibitors; plasminogen activator
86140	C-reactive protein
86141	C-reactive protein; high sensitivity (hsCRP) [2 or more major risk factors, LDL 100-300 mg/dl, and intermediate risk of CVD by global risk assessment
0052U	Lipoprotein, blood, high resolution fractionation and quantitation of lipoproteins, including all five major lipoprotein classes and subclasses of HDL, LDL, and VLDL by vertical auto profile ultracentrifugation
0308U	Cardiology (coronary artery disease [CAD]), analysis of 3 proteins (high sensitivity [hs] troponin, adiponectin, and kidney injury molecule-1 [KIM-1]), plasma, algorithm reported as a risk score for obstructive CAD Proprietary test: HART CAD® Lab/Manufacturer: Prevencio, Inc
0309U	Cardiology (cardiovascular disease), analysis of 4 proteins (NT-proBNP, osteopontin, tissue inhibitor of metalloproteinase-1 [TIMP-1], and kidney injury molecule-1 [KIM-1]), plasma, algorithm reported as a risk score for major adverse cardiac event Proprietary test: HART CVE® Lab/Manufacturer: Prevencio, Inc
0377U	Cardiovascular disease, quantification of advanced serum or plasma lipoprotein profile, by nuclear magnetic resonance (NMR) spectrometry with report of a lipoprotein profile (including 23 variables) Proprietary test: Liposcale® Lab/Manufacturer: CIMA Sciences, 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.

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VIII. Revision History

Revision Date	Summary of Changes
4/26/22	Modified age requirements and updated guidelines referenced in certain sections of criteria; also added CPT codes **0308U** (Cardiology (coronary artery disease [CAD]), analysis of 3 proteins (high sensitivity [hs] troponin, adiponectin, and kidney injury molecule-1 [KIM-1]), plasma, algorithm reported as a risk score for obstructive CAD Proprietary test: HART CAD® Lab/Manufacturer: Prevencio, Inc) and **0309U** (Cardiology (cardiovascular disease), analysis of 4 proteins (NT-proBNP, osteopontin, tissue inhibitor of metalloproteinase-1 [TIMP-1], and kidney injury molecule-1 [KIM-1]), plasma, algorithm reported as a risk score for major adverse cardiac event Proprietary test: HART CVE® Lab/Manufacturer: Prevencio, Inc), which are both not-covered codes with SelectHealth.
8/22/22	Removed all coverage criteria (previous #6, #13, and #14) and all CPT codes (81402, 81403, 81404, 81405, 81406, 81407, 81408, 81479, and 81493) pertaining to genetic testing.
10/24/23	The following changes were implemented: Addition of frequency to coverage criteria #1b, now allowing lipid panel screening on an annual basis for those with increased risk of dyslipidemia; addition of criterion #1bi: "i) Obesity or metabolic syndrome" as a risk factor for annual lipid screening; coverage criteria #3 changed from Lp(a) screening only for those with risk factors to allowing once per lifetime screening of Lp(a): "3) For individuals 18 years of age or older, measurement of lipoprotein a (Lp(a)) once per lifetime MEETS COVERAGE CRITERIA."; addition of frequency for hs-CRP measurement added to coverage criteria #4: "4) For individuals for whom a risk-based treatment decision is uncertain (after quantitative risk assessment using ACC/AHA PCEs to calculate 10-year risk of CVD events [see Note 2]), testing for

Cardiovascular Disease Risk Assessment, continued

	C-reactive protein with the high-sensitivity method (hs-CRP) MEETS COVERAGE CRITERIA at the following frequency: a) For initial screening, two measurements at least two weeks apart. b) If the initial screen was abnormal, follow-up screening is allowed up to once per year."; and for coverage criteria #5 changed "situations" to "cardiovascular disease risk assessments", "hs-CRP" changed to CRP, as no form of CRP (conventional or hs-CRP testing) should be allowed for situations not described in coverage criteria #4.

Celiac Disease Testing

Policy #: AHS – G2043	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 1/8/24 (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

Celiac disease is a hereditary, chronic autoimmune disorder triggered by the ingestion of gluten, a protein found in wheat, rye, and barley. When an individual with celiac disease ingests gluten, the body mounts an immune response that attacks the small intestine. These attacks lead to damage on the villi within the small intestine, inhibiting nutrient absorption (CDF, 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 who have been diagnosed with celiac disease and who are IgA sufficient, serologic testing with IgA anti-tissue transglutaminase (TTG) **MEETS COVERAGE CRITERIA** at the following intervals:

a) At the first follow-up visit 3-6 months after diagnosis.

b) Every 6 months until normalization of anti-TTG levels has occurred.

c) Every 12 to 24 months thereafter.

Celiac Disease Testing, continued

2. For individuals who have been diagnosed with celiac disease and who are IgA deficient, testing for IgG endomysial antibodies, IgG deamidated gliadin peptide, or IgG TTG **MEETS COVERAGE CRITERIA** at the following intervals:

    a) At the first follow-up visit 3-6 months after diagnosis.

    b) Every 6 months until normalization of IgG levels has occurred.

    c) Every 12 to 24 months thereafter.

3. For individuals with signs and symptoms of celiac disease (see Note 1), serologic testing with the IgA anti-TTG and the total IgA test for the diagnosis of celiac disease **MEETS COVERAGE CRITERIA**.

4. For individuals at risk for celiac disease (see Note 1), when IgA anti-TTG is negative or weakly positive, testing for IgA endomysial antibodies **MEETS COVERAGE CRITERIA**.

5. For individuals with clinical suspicion of celiac disease (see Note 1) with an IgA deficiency, testing for IgG endomysial antibodies, IgG deamidated gliadin peptide, or IgG TTG **MEETS COVERAGE CRITERIA**.

6. Testing for IgA and IgG antibodies to deamidated gliadin peptides **MEETS COVERAGE CRITERIA** in any of the following situations:

    a) For individuals under 2 years of age with a clinical suspicion of celiac disease (see Note 1).

    b) For individuals over 2 years of age as a substitute for anti-TTG testing.

7. Genetic testing for HLA DQ2 and DQ8 **MEETS COVERAGE CRITERIA** in any of the following situations:

    a) For symptomatic individuals for whom other testing is undiagnostic.

    b) For symptomatic individuals with positive serology tests who are unable to undergo a biopsy evaluation.

8. For confirmation of celiac disease in individuals at high risk for celiac disease, regardless of the result of celiac disease serology testing, biopsy of the small intestine **MEETS COVERAGE CRITERIA**.

9. Rapid antigen point-of-care testing for anti-TTG **DOES NOT MEET COVERAGE CRITERIA**.

10. Panel testing, multiplex, or multi-analyte testing (for more than two analytes) for the diagnosis or the evaluation of celiac disease **DOES NOT MEET COVERAGE CRITERIA**.

11. For asymptomatic individuals not at an increased risk for developing celiac disease (see Note 1), testing for celiac disease **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.

12. Testing for anti-reticulin antibodies **DOES NOT MEET COVERAGE CRITERIA** for the diagnosis of celiac disease.

13. Testing of stool or saliva samples for the evaluation of celiac disease **DOES NOT MEET COVERAGE CRITERIA.**

14. Serologic testing using an HLA-DQ-gluten tetramer-based assay, including flow cytometry-based HLA-DQ-gluten tetramer assays, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

NOTE 1: Signs and symptoms of celiac disease may include, but are not limited to, the following: unexplained chronic or intermittent diarrhea; unexplained weight loss; unexplained chronic or intermittent abdominal pain or bloating; recurrent nausea or vomiting; unexplained iron deficiency anemia; unexplained vitamin B12 or folate deficiency; unexplained liver transaminase elevations; autoimmune hepatitis; dermatitis herpetiformis; type 1 diabetes; intestinal blockages; unexplained subfertility or miscarriage; unexplained osteoporosis, osteomalacia, or low bone density; and/or primary biliary cirrhosis. Individuals with Down syndrome, Turner syndrome, or Willams-Beuren syndrome are also at high risk for celiac disease. Additionally, in pediatric patients, fatty stools, delayed puberty, amenorrhea, failure to thrive, stunted growth, and/or short stature may also be associated with celiac disease (S. Husby et al., 2020; NICE, 2020; NIDDK, 2016b).

III. Scientific Background

Celiac disease (CD) is an autoimmune disease which occurs due to the body's unfavorable response after the ingestion of gluten. In particular, the body's immune system attacks the small intestine, leading to damage and inhibiting nutrient absorption (CDF, 2018). The clinical presentation of CD is varied and age dependent. In children, failure to thrive, malnutrition, diarrhea, abdominal pain, and distension are common. In adults, abdominal pain, diarrhea or constipation, bloating, and excessive gas are frequent symptoms. Other gastrointestinal symptoms include unexpected weight loss and distension (Kelly, 2023). A high prevalence of CD cases are often found in first degree relatives of CD patients, highlighting genetic aspects of the disease (Nellikkal et al., 2019). Currently, the only treatment for CD is to maintain a gluten-free diet to ameliorate symptoms and improve the quality of life (Caio et al., 2019).

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, 2022) provides the following statistics for CD:

- An estimated 1 in 141 Americans has CD.
- The majority of people are unaware of their status.
- CD can affect all races but is present at a higher rate in Caucasians.
- CD can affect both genders
- CD is more common among people with Down syndrome, Turner syndrome, and type 1 diabetes.

Celiac Disease Testing, continued

- Patients with CD are at risk for Addison's disease, Hashimoto's disease, primary biliary cirrhosis, and type 1 diabetes.

CD has a strong genetic component. The two primary genetic factors for CD susceptibility are the human leukocyte antigen (HLA)-DQ2 and DQ8 alleles (Brown et al., 2019). These genes highlight the role of T cells and the immune response in CD (Tye-Din et al., 2018). Approximately 90-95% of CD patients have the HLA-DQ2 protein encoded by the HLA-DQA1*05 and DQB1*02 alleles. The remaining CD patients have mutations in the HLA-DQ8 protein encoded by the HLA-DQA1*03 and DQB1*03:02 alleles. Stankovic et al. (2014) noted that the absence of susceptible HLA-DQ genotypes makes CD "very unlikely, close to 100%." However, the use of genotyping in diagnosing CD is not without controversy. Paul et al., (2017) report that 25-40% of white Caucasians are positive for the HLA-DQ2/DQ8 haplotype but that only 0.1-1% of the population will develop CD. They also note that the European guidelines released in 2012 recommend genotyping for HLA-DQ2/DQ8 in children with very high anti-TTG titers, but the authors recommend that "HLA-DQ2/DQ8 testing must not be done to 'screen' or 'diagnose' children" with CD (Paul et al., 2017).

Antibodies for the assessment of CD generally fall into one of two categories: autoantibodies (tTG-IgA, anti-endomysial antibody [EMA-IgA]) or antibodies targeting gliadin (DGP-IgA or IgG, antigliadin antibody (AGA)-IgA or IgG). Endomysial antibodies bind to tissue transglutaminase and produce a characteristic staining pattern. Similarly, anti-endomysial antibodies bind to tTG-2, another tissue transglutaminase. The other category of celiac antibodies involve gliadin, which is a component of gluten. Traditional antigliadin antibody tests (AGA-IgA, AGA-IgG) yielded a false positive rate of up to 20%, so they have been replaced with a deamidated gliadin peptide (DGP) (Kelly, 2023).

Genetic testing for HLA DQ2 and DQ8 may also be used to confirm a CD diagnosis. Serologic and histologic HLA-DQ testing requires the patient to be on a gluten-containing diet, which can be a disadvantage to testing. Recently, testing methods for HLA-DQ-gluten tetramer-based assays using flow-cytometry have been developed; these tests can accurately determine whether the patient is on a gluten-containing or gluten-free diet. The assay has a reported 97% sensitivity and 95% specificity for patients on a gluten-free diet as compared to controls (patients without CD) (Sarna et al., 2018). The authors conclude, "This test would allow individuals with suspected celiac disease to avoid gluten challenge and duodenal biopsy, but requires validation in a larger study" (Sarna et al., 2018).

Point of care tests, such as the Simtomax®, have been developed, which detects IgA and IgG antibodies against deamidated gliadin peptides (DGP) and provides a response in just ten minutes (Arenda, 2020). There are also direct-to-consumer (DTC) tests for CD. The FDA-approved 23andme panel includes CD. This test detects a single nucleotide polymorphism in HLA-DQA1 (FDA, 2017).

Clinical Utility and Validity

Olen et al. (2012) evaluated the diagnostic performance and actual costs in clinical practice of immunoglobulin (Ig)G/IgA DGP (deamidated gliadin peptide antibodies) as a complement to IgA-TTG for the diagnosis of pediatric CD. The authors identified 278 children with CD that received a duodenal biopsy. Sensitivity and specificity for tTG were 94% and 86% respectively, but corresponding values for DGP were 91% and 26%. Positive predictive values were 88% for tTG and 51% for DGP. The authors concluded that for diagnosing CD, tTG is superior to DGP, even in children younger than 2 years. Further,

combining tTG and DGP does not provide a better trade-off between number of missed cases of CD, number of unnecessary duodenal biopsies, and cost than utilizing tTG alone (Olen et al., 2012).

Sakly et al. (2012) evaluated the usefulness of anti-DGP antibodies (a-DGP) in the diagnosis of CD. The study included 103 untreated CD patients of all ages and 36 CD patients under a gluten-free diet. The specificity of a-DGP was 93.6% for IgG and 92% for IgA as compared to the 100% for each by anti-endomysium antibodies (AEA) and tTG. The authors concluded that the findings of this study showed "that a-DGP increases neither the sensitivity nor the specificity of AEA and AtTG [anti-tissue transglutaminase antibodies]" (Sakly et al., 2012).

Bufler et al. (2015) evaluated the diagnostic performance of three serological tests for CD. A total of 91 children with CD contributed 411 sera samples and were compared to 98 healthy controls. Transglutaminase type 2(TG2)-IgA, deamidated gliadin peptide (DGP)-IgG, and DGP-IgA were measured. The sensitivity for diagnosis was high for TG2-IgA and DGP-IgG (>90%) but lower for DGP-IgA. Specificity was >97% for all three. Non-adherence to a gluten-free diet was best indicated by positive TG2-IgA. The authors concluded that "combined testing for TG2-IgA and DGP-IgG does not increase the detection rate of CD in IgA competent children compared to TG2-IgA only" (Bufler et al., 2015).

Silvester et al. (2017) performed a meta-analysis to evaluate the "sensitivity and specificity of tTG IgA and EMA [endomysial antibody] IgA assays in identifying patients with celiac disease who have persistent villous atrophy despite a gluten-free diet (GFD)." The authors identified 26 studies for inclusion. The assays were found to have high specificity for identifying patients with persistent villous atrophy (0.83 for tTG IgA, 0.91 for EMA IgA, but with low sensitivity (0.50 for tTG IgA, 0.45 for EMA IgA). No significant difference was seen between pediatric and adult patients. The authors concluded that "we need more-accurate non-invasive markers of mucosal damage in children and adults with celiac disease who are following a GFD" (Silvester et al., 2017).

A report by Selleski et al. (2018) shows that only some of the DQ2/DQ8 alleles were significantly different between pediatric CD patients and pediatric non-CD patients. A total of 97% of the CD patients were positive for at least either DQ2 or DQ8; however, 29.9% of the non-CD patients were also positive for DQ2. In fact, "No significant association was found between DQ2.2 variant and celiac disease in the studied population (Selleski et al., 2018)." Previously, high regard had been given to DQ2.2 variant as being a predisposing variant for CD (Mubarak et al., 2013). Finally, a rapid nucleic acid amplification test using multiplex ligation-dependent probe amplification (MLPA) to detect HLA-DQ2.2, HLA-DQ2.5, and HLA-DQ8 has been developed with a reported 100% specificity for those particular genotypes (Vijzelaar et al., 2016), but this test has not been FDA-approved for use in the United States.

Bajor et al. (2019) performed a meta-analysis focusing on the association between the HLA-DQB1*02 gene doses and the characteristics of CD. The authors identified 24 studies for inclusion in the review and observed that homozygosity of the DQB1*02 allele led to more frequent classical CD (odds ratio [OR] 1.758). The gene dosing effect was more prominent in children (OR: 2.082). Atrophic histology (Marsh grade 3) was more prevalent with a double dose compared to a zero dose (OR: 2.626). No gene dosing effect was seen with diarrhea, age at diagnosis, severity of villous atrophy, or type 1 diabetes. The authors concluded that "A double dose of HLA-DQB1*02 gene seems to predispose patients to

developing classical CD and villous atrophy. Risk stratification by HLA-DQB1*02 gene dose requires further clarification due to the limited available evidence" (Bajor et al., 2019).

Tangermann et al. (2019) completed a prospective study which included 1055 patients all tested for CD with the Simtomax point of care test. The Simtomax detects IgA and IgG antibodies against deamidated gliadin peptides (DGP). All results were compared to the gold standard: histologic analysis of duodenal biopsies. Of all patients who participated in this study, the overall CD prevalence was identified at 4.1%; the Simtomax identified CD with a 79% sensitivity, 94% specificity, 37% positive predictive value, and 99% negative predictive value (Tangermann et al., 2019). When the adult (n=888) and pediatric (n=167) patients were analyzed separately, the Simtomax was found to identify CD with 100% sensitivity and 95% specificity in adults, and 72% sensitivity in children; the authors note that the Simtomax test detected CD with a lower sensitivity than expected (Tangermann et al., 2019).

Profaizer et al. (2020) conducted a study to "evaluate the feasibility of using NGS-based [next-generation sequencing] HLA-B and DQ genotyping for clinical HLA disease association testing and provide direct comparison with the currently used clinical tests, including SSOP [sequence-specific oligonucleotide probe] genotyping, and real-time PCR [polymerase chain reaction] with melting chain analysis." The researchers focused on HLA alleles related to celiac disease, ankylosing spondylitis, abacavir hypersensitivity, carbamazepine hypersensitivity, and allopurinol hypersensitivity. With regards to CD and from 24 samples tested, there was a discrepancy with the *DQB1*03:40* allele with SSOP, real-time PCR, and NGS, but overall, with the different *HLA*-correlations the data has shown "HLA typing by NGS is superior to the existing clinical methods for identifying HLA alleles associated with disease or drug hypersensitivity and offers a viable approach for high volume clinical diagnostic laboratories," continuing to demonstrate the clinical utility of NGS and HLA-testing for CD (Profaizer et al., 2020).

Gould et al. (2021) evaluated CD serologic testing in asymptomatic patients with type 1 diabetes using immunoglobulin A anti-tissue transglutaminase, as there is an increased risk of type 1 diabetes among CD and vice versa. From screening 2,353 patients, the assay with IgA anti-tissue transglutaminase had a positive predictive value of 85.9% when referenced upper limit of normal and had a sensitivity and specificity of 100% and 38%, respectively. This study indicated the need for thresholds for diagnostic evaluation to be population-specific (i.e. to type 1 diabetics), and not taken from the overall population due to the increased risk (Gould et al., 2021).

Schuppan et al. (2021) assessed the efficacy and safety of a 6-weel treatment with ZED1227, a selective oral transglutaminase 2 inhibitor, at three dose levels compared with placebo, in adults with well-controlled celiac disease who underwent a daily gluten challenge. Their primary endpoint was the attenuation of gluten-induced mucosal damage, measured by the ratio of villus height to crypt death. For this study, 41 patients were assigned to the 10-mg ZED1227 group, 41 patients were assigned to the 100-mg group, and 40 patients were assigned to the placebo group. Each had adequate duodenal-biopsy samples for the assessment of the overall endpoint. "The estimated difference from placebo in the change in the mean ratio of villus height to crypt depth from baseline to week 6 was 0.44 (95% confidence interval [CI], 0.15 to 0.73) in the 10-mg group (P = 0.001), 0.49 (95% CI, 0.20 to 0.77) in the 50-mg group (P<0.001), and 0.48 (95% CI, 0.20 to 0.77) in the 100-mg group (P<0.001). The estimated differences from placebo in the change in intraepithelial lymphocyte density were -2.7 cells per 100 epithelial cells (95% CI, -7.6 to 2.2) in the 10-mg group, -4.2 cells per 100 epithelial cells (95% CI, -8.9 to 0.6) in the 50-mg group, and -

9.6 cells per 100 epithelial cells (95% CI, -14.4 to -4.8) in the 100-mg group." The authors concluded that treatment with ZED1227 attenuated gluten-induced duodenal mucosal damage in patients with celiac disease. (Schuppan et al., 2021).

IV. Guidelines and Recommendations

American College of Gastroenterology (ACG)

The ACG recommends to test for CD in the following scenarios (Rubio-Tapia et al., 2013):

1. "Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steatorrhea, postprandial abdominal pain and bloating, should be tested for CD. (Strong recommendation, high level of evidence)"
2. "Patients with symptoms, signs, or laboratory evidence for which CD is a treatable cause should be considered for testing for CD. (Strong recommendation, moderate level of evidence)"
3. "Patients with a first-degree family member who has a confirmed diagnosis of CD should be tested if they show possible signs or symptoms or laboratory evidence of CD. (Strong recommendation, high level of evidence)"
4. "Patients with type I diabetes mellitus should be tested for CD if there are any digestive symptoms, or signs, or laboratory evidence suggestive of celiac disease. (Strong recommendation, high level of evidence)"
5. "Celiac disease should be sought among the explanations for elevated serum aminotransferase levels when no other etiology is found, (Strong recommendation, high level of evidence)"
6. "Consider testing of asymptomatic relatives with a first-degree family member who has a confirmed diagnosis of CD (Conditional recommendation, high level of evidence)"

The ACG guidelines indicate that "Immunoglobulin A (IgA) anti-tissue transglutaminase (TTG) antibody is the preferred single test for detection of CD in individuals over the age of 2 years." Also, if there is "a high probability of CD wherein the possibility of IgA deficiency is considered, total IgA should be measured." Additionally, "an alternative approach is to include both IgA and IgG-based testing, such as IgG-deamidated gliadin peptides (DGPs), in these high-probability patients." In those patients with low or deficient IgA, the ACG recommends "IgG-based testing (IgG DGPs and IgG TTG)." The guidelines also indicate that all serological testing should be done while the individual is on a gluten-containing diet.

Intestinal biopsy is recommended by the ACG for individuals with positive serology testing and for those with a clinical presentation consistent with CD, "even if the serologies are negative."

Although antibodies directed against native gliadin are not recommended for the primary detection of CD," the ACG notes that "when screening children younger than 2 years of age for CD, the IgA TTG test should be combined with DGP (IgA and IgG)."

Celiac Disease Testing, continued

With regard to HLA-DQ2 / DQ8 genotype testing, the ACG recommends that it "should not be used routinely in the initial diagnosis of CD" but rather "should be used to effectively rule out the disease in selected clinical situations" such as, "equivocal small-bowel histological finding (Marsh I-II) in seronegative patients; evaluation of patients on a GFD in whom no testing for CD was done before GFD; patients with discrepant celiac-specific serology and histology; patients with suspicion of refractory CD where the original diagnosis of celiac remains in question; or patients with Down's syndrome… Because HLA-DQ2 is present in approximately 25%–30% of the white population, testing for CD with either HLA-DQ type is not useful because the PPV is only about 12%." Concerning HLA typing, "HLA typing and histological response may help to rule out or confirm the diagnosis of CD in patients with sero-negative CD."

The ACG does not recommend stool or salivary testing, indicating that are not validated for use in the diagnosis of CD.

The ACG advocates monitoring of adherence to a gluten-free diet, based on "a combination of history and serology." Additionally, "upper endoscopy with intestinal biopsies is recommended for monitoring in cases with lack of clinical response or relapse of symptoms despite a GFD."

Celiac Disease Diagnostic Testing Algorithm (Rubio-Tapia et al., 2013)

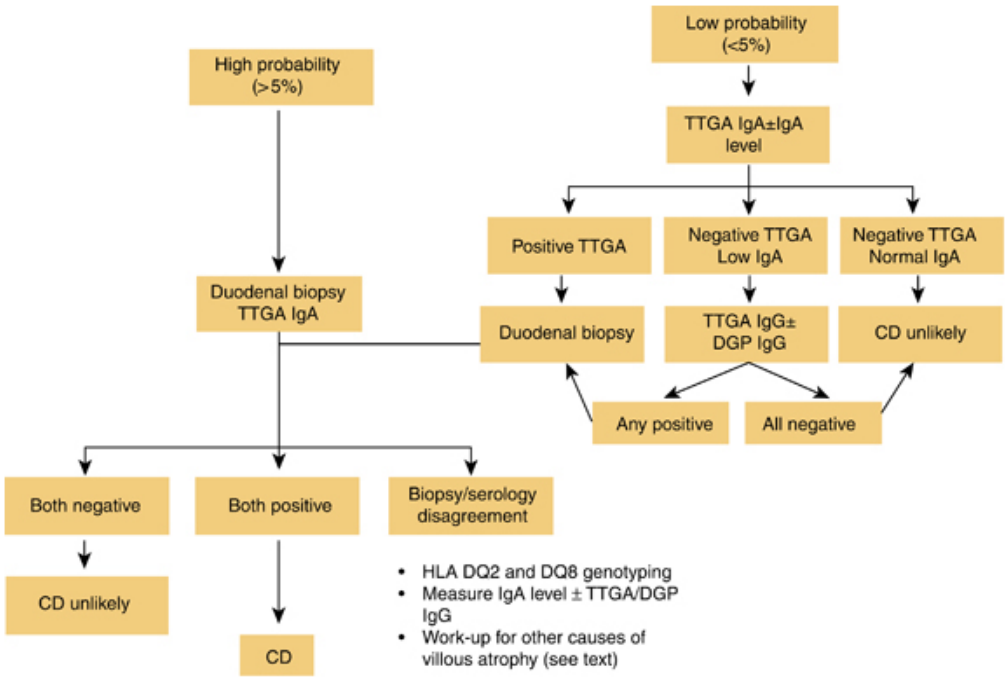

Figure 1. Celiac disease (CD) diagnostic testing algorithm. DGP, deamidated gliadin peptide; HLA, human leukocyte antigen; Ig, immunoglobulin; TTGA, tissue transglutaminase antibody

Celiac Disease Testing, continued

A 2023 update from ACG focused on the diagnosis and management of celiac disease expands upon their previous guidelines:

"1A. We [ACG] recommend EGD with multiple duodenal biopsies for confirmation of diagnosis in both children and adults with suspicion of CD (strong recommendation, moderate quality of evidence; dissent 1).

1B. We suggest a combination of high-level TTG IgA (>10× upper limit of normal) with a positive endomysial antibody (EMA) in a second blood sample as reliable tests for diagnosis of CD in children. In symptomatic adults unwilling or unable to undergo upper GI endoscopy, the same criteria may be considered after the fact, as a diagnosis of likely CD (conditional recommendation, moderate quality of evidence; dissent 0)."

ACG explains the above recommendations by citing key concepts:

"1. Multiple biopsies of the duodenum (1 or 2 from bulb and 4 from distal duodenum) are necessary for diagnosis of CD.

2. EGD and duodenal biopsies can also be useful for the differential diagnosis of other malabsorptive disorders or enteropathies.

3. Lymphocytic duodenosis (≥25 intraepithelial lymphocytes per 100 epithelial cells) in the absence of villous atrophy is not specific for CD, and other causes should be considered" (Rubio-Tapia et al., 2023).

Moreover, in the case of screening procedures, ACG states that

"7A. We recommend case finding to increase detection of CD in clinical practice (strong recommendation, low quality of evidence; dissent 0).

7B. We recommend against mass screening for CD in the community (strong recommendation, low quality of evidence; dissent 0)", on the basis that

"1. Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steatorrhea, abdominal pain, and bloating, should be tested for CD.

2. Patients with symptoms, signs, or laboratory evidence for which CD is a treatable cause should be considered for testing for CD.

3. Patients with a first-degree family member who has a confirmed diagnosis of CD should be tested whether they show possible signs or symptoms or laboratory evidence of CD.

4. Consider testing of asymptomatic relatives with a first-degree family member who has a confirmed diagnosis of CD" (Rubio-Tapia et al., 2023).

Although they note that there is controversy surrounding the best serology approach for children younger than 2 years, ACG

Celiac Disease Testing, continued

"8A. We recommend the immunoglobulin IgA anti-TTG antibody (TTG-IgA) as the preferred single test for the detection of CD in children younger than 2 years who are not IgA-deficient (strong recommendation, moderate quality of evidence; dissent 0).

8B. We recommend that testing for CD in children with IgA deficiency be performed using IgG-based antibodies (DGP-IgG or TTG-IgG) (strong recommendation; moderate quality of evidence; dissent 0)" because

"1. TTG-IgA and EMA-IgA are reported to be less accurate in children younger than 2 years.

2. Current guidelines recommend that testing for CD in children younger than 2 years include both TTG-IgA and DGP-IgG" (Rubio-Tapia et al., 2023).

American Gastroenterological Association (AGA)

Relative to ongoing monitoring of individuals with celiac disease, the AGA recommends periodic serologic testing.

The AGA published an update on CD testing in 2019. Their new "best practice advice" is as follows:

- "Best Practice Advice 1: Serology is a crucial component of the detection and diagnosis of CD, particularly tissue transglutaminase–immunoglobulin A (TG2-IgA), IgA testing, and less frequently, endomysial IgA testing."
- "Best Practice Advice 2: Thorough histological analysis of duodenal biopsies with Marsh classification, counting of lymphocytes per high-power field, and morphometry is important for diagnosis as well as for differential diagnosis."
- "Best Practice Advice 2a: TG2-IgA, at high levels (> ×10 upper normal limit) is a reliable and accurate test for diagnosing active CD. When such a strongly positive TG2-IgA is combined with a positive endomysial antibody in a second blood sample, the positive predictive value for CD is virtually 100%. In adults, esophagogastroduodenoscopy (EGD) and duodenal biopsies may then be performed for purposes of differential diagnosis."
- "Best Practice Advice 3: IgA deficiency is an infrequent but important explanation for why patients with CD may be negative on IgA isotype testing despite strong suspicion. Measuring total IgA levels, IgG deamidated gliadin antibody tests, and TG2-IgG testing in that circumstance is recommended."
- "Best Practice Advice 4: IgG isotype testing for TG2 antibody is not specific in the absence of IgA deficiency."
- "Best Practice Advice 5: In patients found to have CD first by intestinal biopsies, celiac-specific serology should be undertaken as a confirmatory test before initiation of a gluten-free diet (GFD)."
- "Best Practice Advice 6: In patients in whom CD is strongly suspected in the face of negative biopsies, TG2-IgA should still be performed and, if positive, repeat biopsies might be considered either at that time or sometime in the future."
- "Best Practice Advice 7: Reduction or avoidance of gluten before diagnostic testing is discouraged, as it may reduce the sensitivity of both serology and biopsy testing."
- "Best Practice Advice 8: When patients have already started on a GFD before diagnosis, we suggest that the patient go back on a normal diet with 3 slices of wheat bread daily preferably for 1 to 3 months before repeat determination of TG2-IgA."

Celiac Disease Testing, continued

- "Best Practice Advice 9: Determination of HLA-DQ2/DQ8 has a limited role in the diagnosis of CD. Its value is largely related to its negative predictive value to rule out CD in patients who are seronegative in the face of histologic changes, in patients who did not have serologic confirmation at the time of diagnosis, and in those patients with a historic diagnosis of CD; especially as very young children before the introduction of celiac-specific serology" (Steffen Husby et al., 2019).

The AGA's best advice statements for evaluating refractory celiac disease is recorded below.

Best Practice Advice 1

In patients believed to have celiac disease who have persistent or recurrent symptoms or signs, the initial diagnosis of celiac disease should be confirmed by review of prior diagnostic testing, including serologies, endoscopies, and histologic findings.

Best Practice Advice 2

In patients with confirmed celiac disease with persistent or recurrent symptoms or signs (nonresponsive celiac disease), ongoing gluten ingestion should be excluded as a cause of these symptoms with serologic testing, dietitian review, and detection of immunogenic peptides in stool or urine. Esophagogastroduodenoscopy with small bowel biopsies should be performed to look for villous atrophy. If villous atrophy persists or the initial diagnosis of celiac disease was not confirmed, consider other causes of villous atrophy, including common variable immunodeficiency, autoimmune enteropathy, tropical sprue, and medication-induced enteropathy.

Best Practice Advice 3

For patients with nonresponsive celiac disease, after exclusion of gluten ingestion, perform a systematic evaluation for other potential causes of symptoms, including functional bowel disorders, microscopic colitis, pancreatic insufficiency, inflammatory bowel disease, lactose or fructose intolerance, and small intestinal bacterial overgrowth.

Best Practice Advice 4

Use flow cytometry, immunohistochemistry, and T-cell receptor rearrangement studies to distinguish between subtypes of refractory celiac disease and to exclude enteropathy-associated T-cell lymphoma. Type 1 refractory celiac disease is characterized by a normal intraepithelial lymphocyte population and type 2 is defined by the presence of an aberrant, clonal intraepithelial lymphocyte population. Consultation with an expert hematopathologist is necessary to interpret these studies.

Best Practice Advice 5

Perform small bowel imaging with capsule endoscopy and computed tomography or magnetic resonance enterography to exclude enteropathy-associated T-cell lymphoma and ulcerative jejunoileitis at initial diagnosis of type 2 refractory celiac disease.

Best Practice Advice 6

Celiac Disease Testing, continued

Complete a detailed nutritional assessment with investigation of micronutrient and macronutrient deficiencies in patients diagnosed with refractory celiac disease. Check albumin as an independent prognostic factor. (Green et al., 2022)

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)

Updated and expanded evidence-based guidelines for diagnosing CD were published in 2020 by the ESPGHAN. The following recommendations were included (S. Husby et al., 2020):

- "We recommend considering testing for CD in children and adolescents with symptoms, signs and conditions shown in Table 2."
  - Signs and symptoms in Table 2 include:
    - "Gastrointestinal: chronic or intermittent diarrhea, chronic constipation not responding to usual treatment, chronic abdominal pain, distended abdomen, recurrent nausea, recurrent vomiting
    - Extraintestinal symptoms: weight loss, failure to thrive, stunted growth/short stature, delayed puberty, amenorrhea, irritability, chronic fatigue, neuropathy, arthritis/arthralgia, chronic iron-deficiency anemia, decreased bone materialization (osteopenia/osteoporosis), repetitive fractures, recurrent aphthous stomatitis, dermatitis herpetiformis-type rash, dental enamel defects, abnormal liver biochemistry
    - Specific conditions: first-degree relatives with CD, autoimmune conditions: TIDM, thyroid disease, liver disease, Down syndrome, Turner syndrome, Williams-Beuren syndrome, IgA deficiency"
- "HLA- typing does not add to the certainty of the diagnosis if the other criteria for CD diagnosis are fulfilled. Testing for HLA DQ2 and DQ8 may be useful in other circumstances. If no risk alleles are found, CD is unlikely. We recommend that HLA typing is not required in patients with positive TGA-IgA, if they qualify for CD diagnosis with biopsies or if they have high serum TGA-IgA (≥10× ULN) and EMA-IgA positivity. If a patient tests negative for HLA DQ2 and DQ8, the risk of CD is very low, while a positive result does not confirm the diagnosis."
- "Recent studies suggest that the no-biopsy approach to diagnose CD can be applied in asymptomatic children. In asymptomatic children, however, the PPV of high TGA-IgA ≥10× ULN may be lower than in symptomatic children, which needs to be considered during the decision-making process. We give a conditional recommendation that, taking available evidence into account, CD can be diagnosed without duodenal biopsies in asymptomatic children, using the same criteria as in patients with symptoms. We recommend that the decision whether or not to perform diagnostic duodenal biopsies should be made during a shared decision-making process together with the parent(s) and, if appropriate, with the child."
- "The three specific coeliac antibodies (TGA-IgA, EMA-IgA, DGP-IgG) show different performance. TGA-IgA scored highest by a comparison of assay accuracy and is therefore regarded as the most appropriate primary test for CD in the diagnostic work up of children with suspected CD. We recommend that in subjects with normal serum IgA values for age, TGA-IgA should be used as the initial test regardless of age."
- "We recommend testing for total IgA and TGA-IgA as initial screening in children with suspected CD. In patients with low total IgA concentrations, an IgG-based test (DGP, EMA, or TGA) should be

performed as a second step. Testing for EMA, DGP or AGA antibodies (IgG and IgA) as initial screening in clinical practice is not recommended."

- "We recommend that for CD diagnosis without biopsies, TGA-IgA serum concentration of at least 10× ULN should be obligatory. Only antibody tests with proper calibrator curve-based calculation and having the 10× ULN value within their measurement range, should be used. We recommend against omitting biopsies in IgA-deficient cases with positive IgG-based serological tests."
- "We recommend that in children with TGA ≥10X ULN, and parents/patient agreement to the no-biopsy approach, the CD diagnosis should be confirmed by a positive EMA-IgA test in a second blood sample."
- "At least 4 biopsies from the distal duodenum and at least 1 from the duodenal bulb should be taken for histology assessment during a gluten-containing diet. Reading of biopsies should be performed on optimally orientated biopsies. A villous to crypt ratio of <2 indicates mucosal lesions. In cases of discordant results between TGA-IgA results and histopathology, re-cutting of biopsies and/or second opinion from an experienced pathologist should be requested (S. Husby et al., 2020.)"

A 2022 position paper on the management and follow-up of children and adolescents with celiac disease stated that

"3.1 The first follow-up visit should be scheduled 3–6 months after CD diagnosis, but with easy access to the celiac service if earlier advice is needed, and sooner review if there are concerns regarding how the family is coping with the diet, if there are ongoing issues with growth or persistent symptoms or a need to repeat bloodwork earlier. Subsequent visits should be every 6 months until normalization of TGA levels, and every 12–24 months thereafter."

"3.2. During follow-up patients should be evaluated for:

3.2.I. Gastrointestinal and extraintestinal signs and symptoms.

3.2.II. Anthropometric measurements and growth parameters.

3.2.III. IgA-TGA using the same assay as at diagnosis as a surrogate marker for improvement/healing of the small-bowel mucosa. IgG based tests and RIA based IgA-TGA measurements are not suitable for follow-up in IgA sufficient patients. IgA insufficient patients with CD should be followed with IgG based tests.

3.2.IV. A complete blood cell count, micronutritional status (e.g., hemoglobin, iron, vitamin B12, and vitamin D levels) and ALT measurements, should be performed after clinical evaluation at time of diagnosis. Any abnormality should be followed and deficiencies corrected until normalization. If abnormalities persist, additional diagnoses should be considered and appropriately investigated.

3.2.V. Screening for thyroid disease with TSH and thyroxine (and autoantibodies if indicated) may be considered during follow-up after clinical evaluation at the discretion of the clinician.

3.2.VI. Routine bone-density screening is not recommended.

3.2.VII. HBV antibody levels may be measured in previously immunized patients if this is considered important in the population. A booster dose should be given if inadequate levels are present" (Mearin et al., 2022).

A few specific issues of note that may come up during follow-up and management include

"6.1. **How to approach persistent high serum levels of antibodies against tissue-transglutaminase (TGA)?**

Lack of decreasing IgA-TGA levels after 6–12 months on a GFD or persisting positive IgA-TGA levels should be assessed by carefully reviewing dietary compliance and testing IgA-TGA using the same test from the same manufacturer.

6.2. **When is it necessary to (re)biopsy?**

Routine assessment of mucosal healing by small-bowel biopsies is not recommended in children with CD following a GFD. We recommend considering (re) biopsy only in selected CD cases; based on specific clinical grounds, for example, when doubts about the original diagnosis or suspicion of occurrence of an additional condition.

6.3. **Refractory celiac disease in children: does it exist?**

We recommend properly investigating other causes of an apparent "refractory CD" in children, including ongoing inadvertent ingestion of gluten and other possible concomitant enteropathies, such as Crohn's disease, autoimmune enteropathy, small-bowel bacterial overgrowth, cow's milk protein allergy and pancreatic insufficiency" (Mearin et al., 2022).

ESPGHAN suggests that "In cases of uncertain CD diagnosis, HLA typing should be performed before gluten-challenge to detect children in whom the occurrence of CD is unlikely." Moreover, they "recommend the same frequency and follow-up tests in children with CD and T1D as inchildren with isolated CD, with (additional) special attention to test for thyroid involvement and diabetic retinopathy" and that "developing the follow-up plan in conjunction with an endocrinologist/diabetologist and a dietitian, also considering the need for psychological and social support" (Mearin et al., 2022).

In 2012, ESPGHAN recommended that CD testing be considered for: "children and adolescents with the otherwise unexplained symptoms and signs of chronic or intermittent diarrhoea, failure to thrive, weight loss, stunted growth, delayed puberty, amenorrhoea, iron-deficiency anaemia, nausea or vomiting, chronic abdominal pain, cramping or distension, chronic constipation, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetiformis–like rash, fracture with inadequate traumas/osteopenia/osteoporosis, and abnormal liver biochemistry." Testing should also be offered to "asymptomatic children and adolescents with an increased risk for CD such as type 1 diabetes mellitus (T1DM), Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, selective immunoglobulin A (IgA) deficiency, autoimmune liver disease, and first-degree relatives with CD (Husby et al., 2012)."

ESPGHAN recommends that "the initial test be IgA class anti-TG2 from a blood sample. If total serum IgA is not known, then this also should be measured." If the individual has humoral IgA deficiency, "at least 1 additional test measuring IgG class CD-specific antibodies should be done (IgG anti-TG2, IgG anti-DGP or IgG EMA." They also note that "tests measuring antibodies against DGP may be used as additional tests in patients who are negative for other CD-specific antibodies but in whom clinical symptoms raise a strong suspicion of CD, especially if they are younger than 2 years," and "tests for the detection of IgG or IgA antibodies against native gliadin peptides (conventional gliadin antibody test) should not be used for CD diagnosis." They also indicate that "tests for the detection of antibodies of any type in faecal samples should not be used."

For individuals with "severe symptoms and a strong clinical suspicion of CD" and negative serology testing, "small intestinal biopsies and HLA-DQ testing are recommended."

Regarding the evaluation of asymptomatic children and adolescents with CD-associated conditions, ESPGHAN recommends HLA testing "should be offered as the first line test," due to its high negative predictive value. "If the patient is DQ8 and/or DQ2 positive, homozygous for only the bchains of the HLA-DQ2 complex (DQB1_0202), or HLA testing is not done, then an anti-TG2 IgA test and total IgA determination should be performed, but preferably not before the child is 2 years old. If antibodies are negative, then repeated testing for CD-specific antibodies is recommended (S. Husby et al., 2012)."

ESPGHAN also recommends that in asymptomatic individuals at increased genetic risk for CD "duodenal biopsies with the demonstration of an enteropathy should always be part of the CD diagnosis." As an initial step, "it is recommended that the more specific test for EMA be performed. If the EMA test is positive, then the child should be referred for duodenal biopsies. If the EMA test is negative, then repeated serological testing on a normal gluten-containing diet in 3 to 6 monthly intervals is recommended (Husby et al., 2012)." Testing of infants, as with all serologic testing for CD, should be done only when the individual is on a gluten-containing diet.

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

Celiac Disease Testing, continued

NASPGHAN updated their recommendations in 2015 (published in 2016) for gluten-related disorders, including CD, wheat allergy (WA), and nonceliac gluten sensitivity (NCGS). Concerning who should be tested for gluten-related disorders, "Children with symptoms consistent with gluten-related disorders, or who have self-identified relief of symptoms when avoiding gluten, should undergo testing for CD and/or WA before the elimination of dietary gluten. CD should be an early consideration in those with typical gastrointestinal symptoms such as chronic diarrhea, abdominal pain, distension, and weight loss." The table below outlines their recommendations for considering CD testing:

TABLE 2. Indications to consider CD testing

Symptoms	Associated conditions
Abdominal pain	First-degree relatives of those with CD
Abdominal distension	Type 1 diabetes
Diarrhea	Autoimmune thyroid disease
Constipation	Autoimmune liver disease
Growth failure or deceleration	Trisomy 21
Weight loss	Williams syndrome
Arthralgia	Turner syndrome
Elevated hepatic transaminases	IgA deficiency
Iron deficiency anemia	Juvenile chronic arthritis
Unexplained osteopenia	
Dental enamel defects	
Recurrent aphthous stomatitis	
DH	

CD = celiac disease; DH = dermatitis herpetiformis; IgA = immunoglobulin A.

"Children belonging to groups known to be at increased risk for CD may initially have no symptoms, or very minor symptoms, despite having intestinal histologic changes that are characteristic for CD. Included in these groups are first-degree relatives of an index case, people with trisomy 21, Turner syndrome, Williams syndrome, and IgA deficiency, and those with other autoimmune conditions (Hill et al., 2016)."

For initial testing, they recommend the TTG-IgA antibody test due to its reliability and cost-effectiveness. They note that co-testing for serum IgA can be performed to "identify those who have selective IgA deficiency"; however, "use of a panel of antibodies instead of a single tTG-IgA test is not recommended. Although this approach may be associated with a marginal increase in the sensitivity of the test, it

decreases the specificity and significantly increases the costs (Hill et al., 2016)." Testing for serum antibodies against gliadin is less sensitive, reliable, and specific as compared to TTG and EMA.

They do not recommend genetic testing for HLA variants as an initial diagnostic test or screening for CD since up to 40% of the general population contains one of the variant alleles. "Testing for HLA-DQ2/8 is best reserved for patients in whom there is a diagnostic dilemma, such as when there is a discrepancy between the serological and histologic findings or when a GFD [gluten-free diet] has been started before any testing (Hill et al., 2016)."

They do not recommend the use of rapid, point-of-care tests for TTG since these tests do not allow for the quantitative analysis of the antibody.

National Institute for Health and Care Excellence (NICE)

In 2022, NICE published guidance on diagnosing CD. These guidelines state that serological testing should be offered to "people with any of the following: persistent unexplained abdominal or gastrointestinal symptoms, faltering growth, prolonged fatigue, unexpected weight loss, severe or persistent mouth ulcers, unexplained iron, vitamin B12 or folate deficiency, type 1 diabetes, at diagnosis, autoimmune thyroid diseases, at diagnosis, irritable bowel syndrome (in adults), [and] first-degree relatives of people with celiac disease (NICE, 2022)."

"Any test is accurate only if a gluten-free containing diet is eaten during the diagnostic process **and** advise the person not to start a gluten-free diet until diagnosis is confirmed by a specialist, even if the results of a serological test are positive" (NICE,2022).

Further, serological testing for CD could be considered in patients with "metabolic bone disorder (reduced bone mineral density or osteomalacia), unexplained neurological symptoms (particularly peripheral neuropathy or ataxia), unexplained subfertility or miscarriage, persistently raised liver enzymes with unknown cause, dental enamel defects, Down's syndrome, and Turner syndrome (NICE, 2022)."

Finally, regarding serological testing:

- "Test for total IgA and IgA tTG as the first choice
- Use IgA EMA if IgA tTG is weakly positive
- Consider using IgG EMA, IgG DGP or IgG tTG if IgA is deficient (IgA deficiency is defined as total IgA less than 0.07 g per litre) (NICE, 2022)."

In 2015, the National Institute for Health and Care Excellence (NICE) recommended CD serologic testing in symptomatic young people and adults with the following algorithm (NICE, 2015):

- First test for total serum IgA and TTG
- Next test for IgA endomysial antibodies (EMA) if TTG is inconclusive (i.e. weakly positive)
- "Consider using IgG EMA, IgG deamidated gliadin peptide (DGP) or IgG tTG if IgA is deficient"

For children with suspected CD, they recommend:

Celiac Disease Testing, continued

- First test for total serum IgA and TTG
- "Consider using IgG EMA, IgG DGP or IgG tTG if IgA is deficient"

NICE also recommends offer CD testing for people with any of the following:

- Autoimmune thyroid disease
- Persistent unexplained abdominal or gastrointestinal symptoms
- Irritable bowel syndrome
- Type 1 diabetes
- First-degree relatives (parents, siblings or children) with coeliac disease
- Other symptoms indicative of possible CD, including faltering growth in children, prolonged fatigue, unexpected weight loss, severe or persistent mouth ulcers, unexplained dietary deficiencies

NICE also recommends considering CD testing for people with the following:

- Metabolic bone disorder
- Unexplained neurological symptoms
- Unexplained subfertility or recurrent miscarriage
- Down's syndrome or Turner's syndrome
- Dental enamel defects
- Persistent elevated hepatic enzymes of unknown etiology

They do note that "People who are following a normal diet (containing gluten) should be advised to eat gluten in more than 1 meal every day for at least 6 weeks before testing for coeliac disease (NICE, 2016)."

NICE indicates that HLA testing should not be done as part of the initial testing. Also, "Only consider using HLA DQ2 (DQ2.2 and DQ2.5)/DQ8 testing in the diagnosis of coeliac disease in specialist settings (for example, in children who are not having a biopsy, or in people who already have limited gluten ingestion and choose not to have a gluten challenge) (NICE, 2015)."

United States Preventive Services Task Force (USPSTF)

The United States Preventative Services Task Force (Bibbins-Domingo et al., 2017) recently published guidelines on the screening of asymptomatic populations for celiac disease and found that:

"The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for celiac disease in asymptomatic persons. Evidence is lacking, and the balance of benefits and harms cannot be determined." However, it was noted that: "Persons at increased risk for

celiac disease include those who have a positive family history (e.g., a first- or second-degree relative), with an estimated prevalence of 5% to 20%, and persons with other autoimmune diseases (e.g., type 1 diabetes mellitus, inflammatory luminal gastrointestinal disorders, Down syndrome, Turner syndrome, IgA deficiency, and IgA nephropathy). Several specialty societies recommend screening in these populations."

World Gastroenterology Organisation (WGO) Global Guidelines

The WGO published guidelines on CD testing in 2017. A cascade with "resource-sensitive" options is listed.

The "Gold Standard" lists the following items for diagnosis of CD:

- Celiac disease–specific antibodies: assessment and intestinal biopsy
- Anti-tTG IgA or anti-EMA IgA, and total IgA to exclude IgA deficiency
- In case of selective IgA deficiency, IgG-based tests should be used: anti-DGP, anti-tTG, or EMA (the latter 2 are highly sensitive, but with lower specificity)
- Symptomatic patients with a positive serological test or a titer just below the cut-off (borderline) should be referred for endoscopy with multiple duodenal biopsies to confirm or exclude the diagnosis of celiac disease. Pitfalls in histologic diagnosis are common, and findings are characteristic, but not specific
- Asymptomatic patients with a positive serological test should be retested after consuming a gluten-containing diet for 3 months, to confirm persistent seropositivity before referral for endoscopy

The following items are listed for management of CD:

- Follow-up monitoring, including antibody tests (anti-tTG IgA or DGP-IgG in case of IgA deficiency): after 3 to 6 mo in the first year and once a year thereafter in stable patients responding to the gluten-free diet

The WGO also notes that although the presence of HLA risk alleles is "necessary" for celiac disease, it is insufficient for CD development. However, it does have a high negative predictive value, in that absence of those risk alleles excludes CD as a diagnosis.

The WGO notes two main groups of serological markers for untreated CD:

- Autoantibodies targeting the auto-antigen: EMA and anti-tTG antibodies
- Antibodies targeting the offending agent (gliadin): anti- bodies against synthetic deamidated gliadin peptides (anti-DGPs)

Celiac Disease Testing, continued

A summary of the characteristics of CD antibody tests is listed below:

TABLE 8. Sensitivity and Specificity and General Characteristics of Celiac Disease–specific Antibody Tests

Test	Methods	Sensitivity	Specificity	Comments
Autoantibodies against tissue transglutaminase (anti-tTG) IgA	Commercial ELISA, in-house ELISA, RIA, and other methods; cut-off given in arbitrary units, which differ from test to test	Very good	Very good	Autoantibody against autoantigen in celiac disease. Considered by most guidelines as the first-line test for screening, but should be combined with a test based on total IgA or total IgG
Autoantibodies against tissue transglutaminase (anti-tTG) IgG	Commercial ELISA, in-house ELISA, RIA, and other methods, can be combined with an IgA-based test; cut-off given in arbitrary units, which differ from test to test	Very good in IgA-deficient patients Lower in IgA-competent patients	—	Should be used in case of IgA deficiency; combined IgA and IgG-based tests are available
Autoantibodies against endomysium (EMA) IgA	Immunofluorescence on tissue slides; commercially available, results are given in serum dilution with the first positive signal (1:2.5, 1:5, 1:10, etc.) or as positive/negative only	Less sensitive in comparison with anti-tTG IgA	Most specific test; should therefore be used as a confirmatory test if celiac disease diagnosis is made without duodenal biopsies	Requires an immunofluorescence microscope and experienced laboratory technician; more expensive
Autoantibodies against endomysium (EMA) IgG	See EMA IgA Only used in	patients with IgA deficiency	Confirmatory test in patients with IgA deficiency	See EMA IgA
Antibodies against deaminated gliadin peptide (anti-DGP) IgA	Various commercial or in-house ELISA or other methods; cut-off given in arbitrary units, which differ from test to test	Lower in comparison with anti-tTG IgA	Lower in comparison with anti-tTG IgA	May detect patients who are negative for tTG
Antibodies against deaminated gliadin peptide (anti-DGP) IgG	Various commercial or in-house ELISA or other methods; cut-off given in arbitrary units, which differ from test to test	Very good. Alternative test to anti-tTG IgG in patients with IgA deficiency	Slightly lower in comparison with anti-tTG IgA	A high rate of false-positive results has been reported in infants; may detect patients negative for tTG

Anti-DGP indicates antibodies to deaminated gliadin peptides; anti-tTG, antibodies to tissue transglutaminase-2; ELISA, enzyme-linked immunosorbent assay; EMA, endomysial antibodies; IgA, immunoglobulin A; IgG, immunoglobulin G; RIA, radioimmunoassay.

The WGO also lists several conditions associated with a higher risk of CD. Those conditions are as follows:

- Type 1 diabetes mellitus
- Autoimmune thyroid disease
- Autoimmune liver disease
- Down syndrome
- Turner syndrome
- Williams syndrome
- Selective IgA deficiency
- Unexplained elevated serum aminotransferase levels

The WGO also recommends that first-degree relatives of index (affected) patients to be screened for CD.

Finally, WGO recommends against use of urine, stool, or saliva measurements in clinical practice, as they have a "lower performance" than blood-based tests (Bai & Ciacci, 2017).

European Society for the Study of Coeliac Disease (ESsCD)

The ESsCD published guidelines on CD, including recommendations on serological and genetic testing. These recommendations are listed below:

- "Adult patients with symptoms, signs or laboratory evidence suggestive of malabsorption should be tested with serology for CD. (Strong recommendation, high level of evidence)"
- "Screening of asymptomatic first-degree family member of CD patient is recommended. If available, HLA-typing may be offered as the first-line test; if negative, no further work-up is needed. (Conditional recommendation, high level of evidence)"
- "CD should be excluded in patients with unexplained elevation of serum aminotransferase levels. (Strong recommendation, high level of evidence)"
- "T1DM should be screened regularly for CD. (Strong recommendation, high level of evidence)"
- "IgA-TG2 antibody is the preferred single test for detection of CD at any age. (Strong recommendation, high level of evidence)"
- "Total IgA level needs to be measured concurrently with serology testing to determine whether IgA levels are sufficient. (Strong recommendation, moderate level of evidence)"
- "In patients with selective total IgA-deficiency, IgG-based testing (IgG-DGPs or IgG-TG2) should be performed at diagnosis and follow-up. (Strong recommendation, moderate level of evidence)"
- "All diagnostic serologic testing should be done while patients on a gluten-containing diet. (Strong recommendation, high level of evidence)"
- "Antibodies directed against native gliadin (AGA) are not recommended for the primary detection of CD. (Strong recommendation, high level of evidence)"
- "Intestinal-permeability tests are neither sensitive nor specific and are not recommended for CD diagnosis. (Strong recommendation, moderate level of evidence)"
- "Serum I-FABP might be useful in identifying dietary non-adherence and unintentional gluten intake. (Strong recommendation, moderate level of evidence)"
- "A newly diagnosed adult CD patient should undergo testing to uncover deficiencies of essential micronutrient, e.g. iron, folic acid, vitamin D and vitamin B12. (Strong recommendation, moderate level of evidence)"
- "CD diagnosis may be made without duodenal biopsy in symptomatic children with high TG2 levels (>10 times ULN) and EMA in the presence of HLA-DQ2/8. The diagnosis is confirmed by an antibody decline and preferably a clinical response to a GFD". (Conditional recommendation, moderate level of evidence)

The ESsCD also lists recommendations for HLA-DQ2/8 typing, which are as follows:

- "A negative HLA test is helpful to exclude the possibility of CD. This is especially helpful in those already on a GFD before testing."
- "When diagnosis of CD is uncertain, e.g., negative serology, but histology suggestive of CD."
- "To distinguish siblings who can be reassured that it is unlikely that they will develop CD from those who need to be monitored. Furthermore, the data on the quality of life on a GFD in those patients

detected by screening are conflicting, but there is a trend towards improvement. Also, the lack of understanding of the natural history of undiagnosed CD may justify screening asymptomatic persons."

- "In subjects with other autoimmune diseases and some genetic disorders who should be investigated for CD."
- "HLA-DQ2/DQ8 testing should not be used routinely in the initial diagnosis of CD. It is recommended that the results of such testing should be included along with a caution that patients at risk should be serologically tested for CD without changing their diet. (Strong recommendation, moderate level of evidence) (Al-Toma et al., 2019)."

British Society of Gastroenterology (BSG)

In 2014 the BSG published guidelines for the diagnosis and management of adult CD. The following guidelines were included:

- "Diagnosis of CD requires duodenal biopsy when the patient is on a gluten-containing diet and for the vast majority of adult patients also positive serology. (Grade B)
- Biopsy remains essential for the diagnosis of adult CD and cannot be replaced by serology. Follow-up should aim at strict adherence to a gluten-free diet. (Grade B)
- In individuals undergoing an upper endoscopy in whom laboratory tests or symptoms or endoscopic features suggest CD, duodenal biopsy should be considered. (Grade C)
- HLA typing should be used to rule out CD. A positive DQ2.5 or DQ8 can never confirm the diagnosis. (Grade B)
- HLA typing should be used in individuals who are self-treated on a GFD and never had appropriate testing for CD before changing their diet. (Grade B)
- HLA typing can be used to rule out CD, and minimise future testing, in high-risk individuals with CD, for example, first-degree relatives. (Grade B)
- The diagnosis of CD requires duodenal biopsy when the patient is on a gluten-containing diet and for the vast majority of adult patients also positive serology. (Grade B)
- Duodenal biopsy should be retained as the mainstay for the diagnosis of adult CD and cannot be replaced by serology. (Grade B)
- At endoscopy, if there is suspicion of CD, then at least four biopsy specimens should be obtained, including a duodenal bulb biopsy. (Grade C)
- In serologically negative patients showing signs of malabsorption (such as anaemia or diarrhoea) or a family history of CD, a duodenal biopsy should be considered. (Grade C)
- Follow-up biopsies may be considered in patients with CD, and are potentially helpful in identifying patients at increased risk of lymphoma. (Grade B) (Ludvigsson et al., 2014)"

British Society of Paediatric Gastroenterology, Hepatology, and Nutrition (BSPGHAN) and Coeliac UK

In 2013, the BSPGHAN and Coeliac UK published joint guidelines for the management of CD in children. These guidelines note that "Anti-tTG antibody positivity alone is insufficient for diagnosis. Therapeutic trials of GFD are NOT indicated if CD is suspected." Further, if the patient is symptomatic, IgA and IgA tTG should be checked first" (Murch et al., 2013).

Celiac Disease Testing, continued

- "If tTG negative and IgA normal, CD unlikely: If IgA low, then further testing (e.g., IgG tTG and possible biopsy) is required.
- If tTG raised—but less than 10×upper limit of normal for assay: Duodenal biopsy is required. At endoscopy, take four biopsies from D2 or lower and 1–2 from duodenal bulb (as patchy changes may be present). Ensure adequate gluten intake prior to testing with advice from dietician if necessary
- If tTG raised—and greater than 10×upper limit of normal for assay: Take further blood sample to check IgA-EMA and determine HLA-DQ2/HLA-DQ8 typing. If EMA+ and patient either DQ2 or DQ8, the diagnosis is confirmed without the need for a duodenal biopsy. If EMA antibody testing is not locally available, a second strongly positive tTG antibody may be substituted and serum saved for later EMA testing (Murch et al., 2013)."

Society for the Study of Celiac Disease (SSCD)

In 2017, the NASSCD uploaded a guideline for celiac disease diagnosis in adults, along with a diagram detailing the algorithm for a positive celiac disease diagnosis with presenting GI and/or extraintestinal symptoms and signs. They state:

"Celiac disease (CD) may be suspected in

1. Symptomatic patients with
   - Gastrointestinal symptoms/signs: diarrhea, weight loss, gas/bloating, constipation (more commonly in children), hypertransaminasemia
   - Extraintestinal symptoms/signs: iron deficiency anemia, dermatitis herpetiformis, osteoporosis and neuropsychiatric conditions, such as neuropathy or ataxia
2. Patients with associated conditions
   - Type 1 diabetes mellitus
   - Autoimmune thyroiditis
   - Other autoimmune conditions
   - Down syndrome
3. First-degree family members of celiac patients[a]"

Cervical Cancer Screening

Policy #: AHS – G2002	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 2/13/23, 1/8/24 (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

Cervical cancer screening detects cervical precancerous lesions and cancer through cytology, human papillomavirus (HPV) testing, and if needed, colposcopy (Feldman,et al., 2022). The principal screening test to detect cancer in asymptomatic women is the Papanicolaou (Pap) smear. It involves cells being scraped from the cervix during a pelvic examination and spread onto a slide. The slide is then sent to an accredited laboratory to be stained, observed, and interpreted (Feldman & Crum, 2022).

Human papilloma virus (HPV) has been associated with development of cervical intraepithelial neoplasia, and FDA approved HPV tests detecting the presence of viral DNA from high risk strains have been developed and validated as an adjunct primary cancer screening method (Feldman & Crum, 2019).

For more information specifically regarding HPV, please refer to AHS-G2157 Diagnostic Testing of STIs.

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.

The criteria below are based on recommendations by the U.S. Preventive Services Task Force, The National Cancer Institute, NCCN, The American Society for Colposcopy and Cervical Pathology, The

Cervical Cancer Screening, continued

American Cancer Society, The American Society for Clinical Pathology, and the American College of Obstetricians and Gynecologists. Within these coverage criteria, "individual(s)" is specific to individuals with a cervix.

1. For immunosuppressed individuals, any one of the following cervical cancer screening techniques **MEETS COVERAGE CRITERIA:**

a. Annual cervical cytology testing for individuals less than 30 years of age.

b. Co-testing (cervical cytology and HPV) once every 3 years for individuals 30 years of age or older.

2. For individuals 21 to 29 years of age, cervical cancer screening once every 3 years using conventional or liquid based Papanicolaou (Pap) smears **MEETS COVERAGE CRITERIA**.

3. For individuals 30 to 65 years of age, any one of the following cervical cancer screening techniques **MEETS COVERAGE CRITERIA:**

a) Conventional or liquid-based Pap smear once every 3 years.

b) Cervical cancer screening using the high-risk HPV test alone once every 5 years.

c) Co-testing (cytology with concurrent high-risk HPV testing) once every 5 years.

4. For individuals who are over 65 years of age and who are considered high-risk (individuals with a high-grade precancerous lesion or cervical cancer, individuals with in utero exposure to diethylstilbestrol, or individuals who are immunocompromised), cervical cancer screening at the frequency described in coverage criterion 3 **MEETS COVERAGE CRITERIA.**

5. For individuals who are HPV positive and cytology negative, testing for high-risk strains HPV-16 and HPV-18 **MEETS COVERAGE CRITERIA.**

6. Annual cervical cancer screening by Pap smear or HPV testing in one year **MEETS COVERAGE CRITERIA** in the following situations:

a. For individuals who had a previous cervical cancer screen with an abnormal cytology result and/or who was positive for HPV.

b. For individuals at high risk for cervical cancer (organ transplant, exposure to the drug DES, immunocompromised individuals).

7. For individuals over 65 years of age who are not considered high-risk and who have an adequate screening history, routine cervical cancer screening **DOES NOT MEET COVERAGE CRITERIA.** Adequate screening history is defined as either:

a) Having three consecutive negative Pap smears.

b) Having two consecutive negative HPV tests within 10 years before cessation of screening, with the most recent test occurring within 5 years.

9. For individuals who have undergone surgical removal of the uterus and cervix and who have no history of cervical cancer or pre-cancer, cervical cancer screening (at any age) **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.

10. The following **DO NOT MEET COVERAGE CRITERIA:**

a. Inclusion of low-risk strains of HPV in co-testing.

b. Other technologies for cervical cancer screening.

III. Scientific Background

The American Cancer Society estimates that 13,960 new cases of cervical cancer will be diagnosed in 2023 and approximately 4,310 of these individuals will die from the disease (ACS, 2023). To screen for cervical cancer, a Papanicolaou (Pap) test or human papillomavirus (HPV) test is performed. Co-testing with both is also a common clinical practice. To obtain the cell sample for cytology, cells are scraped from both the ectocervix (external surface) and endocervix (cervical canal) during a speculum exam to evaluate the squamocolumnar junction where most neoplasia occur. Cytological examination can be performed as either a traditional Pap smear where the swab is rolled directly on the slide for observation or as a liquid-based thin layer cytology examination where the swab is swirled in a liquid solution so that the free cells can be trapped and plated as a monolayer on the glass slide. One advantage of the liquid cytology assay is that the same sample can be used for HPV testing whereas a traditional Pap smear requires a second sample to be taken. HPV testing is typically a nucleic acid-based assay that checks for the presence of high-risk types of HPV, especially types 16 and 18. HPV testing can be performed on samples obtained during a cervical exam; furthermore, testing can be performed on samples obtained from a tampon, Dacron or cotton swab, cytobrush, or cervicovaginal lavage (Feldman & Crum, 2022).

Cervical cancer screening recommendations for average-risk individuals generally fall into categories based on an individual's age (William R Robinson, 2023b):

- Age < 21 – It is suggested to not screen for cervical cancer in asymptomatic and immunocompetent patients (as observational studies show a low incidence and benefits may outweigh the harms of false positives).
- Age 21 to 29 – In average patients that are asymptomatic and immunocompetent, the age at which to initiate screening is contested and the ideal testing method varies by guideline. Opinions for expert groups also vary. A preference for cytology (rather than HPV testing) for this subgroup is based on a meta-analysis of randomized trials that revealed higher false positive rates for HPV testing.
- Age 30 to 65 – It is recommended that cervical cancer screening continues in all immunocompetent and asymptomatic individuals with a cervix. The methods range from primary HPV testing every 5 years to co-testing (Pap and HPV testing) every five years; or a Pap test alone every three years.
- Age >65 years – The decision to halt cervical cancer screening in asymptomatic and immunocompetent patients can depend on factors such as prior screening results, life expectancy, and patient preference, but it is suggested to discontinue screening for this subgroup if there has been adequate prior screening.

The above recommendations do not account for special populations such as patients with HIV, immunosuppression, and in utero exposure to diethylstilbestrol (DES). These populations are at greater risk for developing cervical cancer (William R Robinson, 2023b).

The following are the initial screening recommendations for individuals with HIV (William R Robinson, 2023a):

- Initial screening for HIV should occur when HIV is first diagnosed (but at no earlier than 21 years of age).
- Age 21 to 29 – Cervical cytology is the preferred method for screening.
- Age 30 years or older – Cervical cytology or co-testing are both appropriate. However, the use of HPV testing alone (i.e., without co-testing) is NOT recommended for this subgroup.

For patients with HIV in whom initial screening is normal, subsequent screening is categorized based upon method (i.e., cervical cytology, co-testing, colposcopy)(William R Robinson, 2023a):

- Cervical cytology: Those screened with cervical cytology (patients 21 to 29 years and those 30 and older) should have cervical cytology performed every 12 months for a total of three years. If results of three consecutive cytology tests are normal, a follow-up test can occur every three years.
- Co-testing: Those screened with co-testing (30 years and older) should have this co-testing occur every three years.
- Colposcopy: Should not be performed routinely at follow-up visits.
- Screening in the HIV population should occur throughout a patient's lifetime and should not stop at 65 years old (contrasted against the general average patient recommendations, which suggest discontinuing at 65 years old).

Analytical Validity

A study by Marchand et al. (2005) explored the optimal collection technique for Pap testing. Their study occurred in two different cytology labs and 128 clinicians participated in the study over the course of one year. The authors discovered that in conventional Pap testing the sequence of collection—the cytobrush for the endocervix and the spatula for the ectocervix—had no effect on the quality of the assay. Further, 47% of the clinicians who had high levels of absent endocervical cells on their samples used the cytobrush method alone. The authors conclude, "The combination of the Cytobrush (endocervix) and spatula (ectocervix) is superior for a quality Pap smear. The sequence of collection was not important in conventional Pap smears. The broom alone performs poorly (Marchand et al., 2005)."

Urine-based HPV DNA testing as a screening tool would be a less invasive method than cervical examinations and swabs. A study by Mendez et al. (2014) using both urine samples and cervical swabs from 52 patients, however, showed that there was only 76% agreement between the two methodologies. The urine testing correctly identified 100% of the uninfected individuals but only 65% of the infected as compared to the cervical swab controls (Mendez et al., 2014). An extensive meta-analysis of 14 different studies using urinary testing, on the other hand, reported an 87% sensitivity and 94% specificity of the urine-based methodology for all strains of HPV, but the sensitivity for high-risk

strains alone was only 77%. The specificity for the high-risk strains alone was reported to be higher at 98%. “The major limitations of this review are the lack of a strictly uniform method for the detection of HPV in urine and the variation in accuracy between individual studies. Testing urine for HPV seems to have good accuracy for the detection of cervical HPV, and testing first void urine samples is more accurate than random or midstream sampling. When cervical HPV detection is considered difficult in particular subgroups, urine testing should be regarded as an acceptable alternative (Pathak et al., 2014).”

Clinical Utility and Validity

The National Cancer Institute (NCI) reports that “Regular Pap screening decreases cervix cancer incidence and mortality by at least 80%” (NCI, 2023). They also note that Pap testing can result in the possibility of additional diagnostic testing, especially in younger individuals, when unwarranted, especially in cases of possible low-grade squamous intraepithelial lesions (LSILs); however, even though 50% of individuals undergoing Pap testing required additional, follow-up diagnostic procedures, only 5% were treated for LSILs. The NCI also reports that “HPV-based screening provides 60% to 70% greater protection against invasive cervical carcinoma, compared with cytology” (NCI, 2023).

A study by Sabeena et al. (2019) measured the utility of urine-based sampling for cervical cancer screening in low-resource settings. The researchers compared 114 samples to determine the accuracy of HPV detection (by polymerase chain reaction (PCR)) in paired cervical and urine samples. Samples were taken from patients previously diagnosed with cervical cancer through histological methods. Of the 114 samples, “HPV DNA was tested positive in cervical samples of 89 (78.1%) and urine samples of 55 (48.2%) patients. The agreement between the two sampling methods was 66.7%” (Sabeena et al., 2019). HPV detection in urine samples had a sensitivity of 59.6% and a specificity of 92%. The authors concluded, “Even though not acceptable as an HPV DNA screening tool due to low sensitivity, the urine sampling method is inexpensive and more socially acceptable for large epidemiological surveys in developing countries to estimate the burden” (Sabeena et al., 2019).

Cervical cancer guidelines published by the National Comprehensive Cancer Network (NCCN) (NCCN, 2023) state that, although the rates of both incidence and mortality of squamous cell carcinoma of the cervix have been declining over the last thirty years, “adenocarcinoma of the cervix has increased over the past 3 decades, probably because cervical cytologic screening methods are less effective for adenocarcinoma.” A study in the United Kingdom supports this increase in adenocarcinoma findings because the risk-reduction associated with three yearly screenings was reduced by 75% for squamous carcinoma and 83% for adenosquamous carcinoma, but adenocarcinoma was reduced only by 43% (Sasieni et al., 2009). Another extensive study of more than 900,000 individuals in Sweden showed that PCR-based HPV testing for the high-risk types 16 and 18 is better at predicting the risk of both in situ and invasive adenocarcinoma. The authors conclude, “infections with HPV 16 and 18 are detectable up to at least 14 years before diagnosis of cervical adenocarcinoma. Our data provide prospective evidence that the association of HPV 16/18 with cervical adenocarcinoma is strong and causal (Dahlstrom et al., 2010).”

Cervical Cancer Screening, continued

A report by Chen et al. (2011) reviewed HPV testing and the risk of the development of cervical cancer. Of the 11,923 individuals participating in the study, 86% of those who tested positive for HPV did not develop cervical cancer with ten years. The authors concluded, "HPV negativity was associated with a very low long-term risk of cervical cancer. Persistent detection of HPV among cytologically normal women greatly increased risk. Thus, it is useful to perform repeated HPV testing following an initial positive test (Chen et al., 2011)."

In 2018, the results of a multi-year cervical cancer screening trial (FOCAL) were published. This randomized clinical trial test of the use of HPV testing alone for detection of cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+). More than 19,000 individuals participated in the study split between the intervention group (HPV testing alone) and the control group (liquid-based cytology). Among individuals who underwent cervical cancer screening, the use of primary HPV testing as compared with cytology testing resulted in a significantly lower likelihood of CIN3+ at 48 months. "Further research is needed to understand long-term clinical outcomes as well as cost-effectiveness" (Ogilvie et al., 2018). In a commentary concerning the findings of this trial, the author noted that "multiple randomized trials have shown that primary HPV screening linked to subsequent identification and treatment of cervical precancer is more effective than Pap testing in reducing the incidence of cervical cancer and precancer, at the cost of lower specificity and more false-negative subsequent colposcopic assessments (Massad, 2018)." The author did not address the limitations of the FOCAL study, including that the study concluded prior to seeing what effects, if any, those vaccinated against HPV 16 and HPV 18 would have since the adolescents vaccinated upon FDA approval of the vaccine would not have necessarily been included within the study. They also state that a limitation of the FOCAL trial is "the use of a pooled HPV test for screening, incorporating all carcinogenic HPV types in a single positive or negative result" (Massad, 2018).

Melnikow et al. (2018) performed a review for the USPSTF regarding cervical cancer screening through high-risk (hr) HPV testing. The authors reviewed the following studies: "8 randomized clinical trials (n = 410556), 5 cohort studies (n = 402615), and 1 individual participant data (IPD) meta-analysis (n = 176464)." Primary hr-HPV testing was found to detect cervical intraepithelial neoplasia (CIN) 3+ at an increased rate (relative risk rate ranging from 1.61 to 7.46) in round 1 screening. False positive rates for primary hr-HPV testing ranged from 6.6% to 7.4%, compared with 2.6% to 6.5% for cytology, whereas in cotesting, false-positives ranged from 5.8% to 19.9% in the first round of screening, compared with 2.6% to 10.9% for cytology. Overall, the authors concluded that "primary hrHPV screening detected higher rates of CIN 3+ at first-round screening compared with cytology. Cotesting trials did not show initial increased CIN 3+ detection (Melnikow et al., 2018)."

Bonde et al. (2020) performed a systematic review on the clinical utility of HPV genotyping as a form of cervical cancer screening. Through 16 studies, the researchers concluded that "HPV genotyping can refine clinical management" for individuals "screened through the primary HPV paradigm and the co-testing paradigm by stratifying genotype-specific results and thereby assign women at highest risk for cervical disease to further testing (i.e., colposcopy) or treatment, while designating those with lowest risk to retesting at a shortened interval." After deeming low risk of bias, the review also stated "the overall quality of evidence for CIN 3 or worse risk with negative for intraepithelial lesions or

malignancies or low-grade squamous intraepithelial cytology was assessed as moderate; that with atypical squamous cells-undetermined significance and "all cytology" was assessed as high… Human papillomavirus genotyping discriminated risk of CIN 3 or worse to a clinically significant degree, regardless of cytology result" (Bonde et al., 2020).

Between 2010 and 2019, Pry et al. (2021) reviewed 204,225 results from 183,165 study participants across 11 government health facilities in Lusaka, Zambia, as part of the Cervical Cancer Prevention Program in Zambia (CCPPZ). By examining precancerous lesions via visual inspection with acetic acid and digital cervicography (VIAC), they were "able to show that the highest odds for screening positive are among women aged 20–29 years" and that "women in the 30–39 years age group had the highest proportion of positive screening results (11·3%) among those with age recorded"; interestingly, however, "Women who were HIV-positive and younger than 20 years had more than three times the predictive probability (18·4, 95% CI 9·56–27·32) for being positive compared with women who were HIV-negative in the same age group (predictive probability 5·5%, 95% CI 3·2–7·8)" (Pry et al., 2021). But while the high proportion of the screen positivity in individuals younger than 20 years old may suggest "that women with HIV have earlier disease progression" and "that these women should be engaged in screening at a younger age", these data could be the result of "some misalignment between screening test positivity and neoplastic lesions, as visually, cervicitis and other benign cervical lesions could be mistaken for pre-cancerous disease" or even simply the inherent weaknesses in the test accuracy of the VIAC method ("sensitivity from 25% (95% CI 7–59) to 82% (66–95) and specificity from 74% (64–82) to 83% (77–87)"), warranting further examination (Pry et al., 2021).

Many guidelines call for the cessation of cervical cancer screening after the age of 65; however, Dilley et al. (2021) argues for a reevaluation of recommendations of this ilk, given that 20% of new cervical cancers occur in this group. Moreover, elderly individuals with a cervix are not only more likely to be diagnosed with late-stage cancer, but also receive commensurately worse outcomes and higher mortality rates. The authors point to the use of theoretical modelling and expert opinion as leading drivers of misconceptions about cervical screening harm in older individuals, specifying that while many of the models seek to minimize the harms and costs associated with increased colposcopies, they are remiss in their consideration of the costs and benefits of "the treatment of advanced cancer, such as cold knife conization, radical hysterectomy, pelvic radiation therapy and chemotherapy" and in their interpretation of exiguous data on the benefits and harms of screening after 65. Furthermore, though the existing guidelines suggest that "the guidelines account for the importance of adequate prior screening before cessation of screening, as the majority of cervical cancer cases are diagnosed in women who have not been adequately screened", the authors counter that "studies have shown that only 25–50% of women diagnosed with cervical cancer had "adequate prior screening" before their cancer diagnosis, which will only be further exacerbated as the population continues to age (Dilley et al., 2021).

V. Guidelines and Recommendations

U.S. Preventive Services Task Force (USPSTF)

The USPSTF updated their recommendations in 2018. The recommendations are outlined in the table below. The USPSTF changed the recommendation concerning women aged 30-65 to now include the possibility of high-risk HPV testing alone once every five years as a screening. They still allow for the possibility of co-testing every five years or for Pap testing alone every three years.

Cervical Cancer Screening, continued

The USPSTF notes certain risk factors that may increase the risk of cervical cancer, such as "HIV infection, a compromised immune system, in utero exposure to diethylstilbestrol, and previous treatment of a high-grade precancerous lesion or cervical cancer." Cytology, primary testing for high-risk HPV alone, or both methods simultaneously may detect the high-risk lesions that are precursors to cervical cancer (USPSTF, 2018).

ACOG endorses these recommendations from the USPSTF (ACOG, 2018a).

USPSTF Summary of Recommendations and Evidence (USPSTF, 2018b)

Population	Recommendation	Grade
Women 21 to 65 years of age	For women 21 to 29 years of age, screen for cervical cancer every 3 years with cytology alone. For women 30 to 65 years of age, screen for cervical cancer every 3 years with cytology alone, every 5 years with high-risk (hr) HPV testing alone, or every 5 years with co-testing.	The USPSTF recommends the service. There is high certainty that the net benefit is substantial. Offer or provide this service. Grade A
Women younger than 21, older than 65, who have had adequate prior screening, or who have had had a hysterectomy	Do not screen for cervical cancer.	The USPSTF recommends against the service. There is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits. Discourage the use of this service. Grade D

In 2017, "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of performing screening pelvic examinations in asymptomatic, nonpregnant adult women. (I statement) This statement does not apply to specific disorders for which the USPSTF already recommends screening (i.e., screening for cervical cancer with a Papanicolaou smear, screening for gonorrhea and chlamydia)."

National Comprehensive Cancer Network (NCCN)

Regarding the diagnosis and workup for cervical cancer, the NCCN states that "The earliest stages of cervical carcinoma may be asymptomatic or associated with a watery vaginal discharge and postcoital bleeding or intermittent spotting. Often these early symptoms are not recognized by the patient. Because of the accessibility of the uterine cervix, cervical cytology or Papanicolaou (Pap) smears and cervical biopsies can usually result in an accurate diagnosis. Cone biopsy (ie, conization) is recommended if the

cervical biopsy is inadequate to define invasiveness or if accurate assessment of microinvasive disease is required. However, cervical cytologic screening methods are less useful for diagnosing adenocarcinoma, because adenocarcinoma in situ affects areas of the cervix that are harder to sample (i.e., endocervical canal)" and that "Workup for these patients with suspicious symptoms includes history and physical examination, complete blood count (CBC; including platelets), and liver and renal function tests" (NCCN, 2023).

The NCCN also remarked that "Persistent human papillomavirus (HPV) infection is the most important factor in the development of cervical cancer. The incidence of cervical cancer appears to be related to the prevalence of HPV in the population. Screening methods using HPV testing may increase detection of adenocarcinoma" , adducing that "In developed countries, the substantial decline in incidence and mortality of squamous cell carcinoma of the cervix is presumed to be the result of effective screening, although racial, ethnic, and geographic disparities exist" (NCCN, 2023). As such, the NCCN lists chronic, persistent HPV infection along with persistently abnormal Pap smear tests as criteria to be considered for women contemplating hysterectomy.

National Cancer Institute (NCI)

Concerning the use of Pap testing in screening, the NCI recommends: "Based on solid evidence, regular screening of appropriate women for cervical cancer with the Pap test reduces mortality from cervical cancer. The benefits of screening women younger than 21 years are small because of the low prevalence of lesions that will progress to invasive cancer. Screening is not beneficial in women older than 65 years if they have had a recent history of negative test results… Based on solid evidence, regular screening with the Pap test leads to additional diagnostic procedures (e.g., colposcopy) and possible overtreatment for low-grade squamous intraepithelial lesions (LSILs). These harms are greatest for younger women, who have a higher prevalence of LSILs, lesions that often regress without treatment. Harms are also increased in younger women because they have a higher rate of false-positive results. Excisional procedures to treat preinvasive disease has been associated with increased risk of long-term consequences for fertility and pregnancy" (NCI, 2023).

Concerning the use of HPV DNA testing, the NCI states: "Based on solid evidence, screening with the HPV DNA or HPV RNA test detects high-grade cervical dysplasia, a precursor lesion for cervical cancer. Additional clinical trials show that HPV testing is superior to other cervical cancer screening strategies. In April 2014, the U.S. Food and Drug Administration approved an HPV DNA test that can be used alone for the primary screening of cervical cancer risk in women aged 25 years and older… Based on solid evidence, HPV testing identifies numerous infections that will not lead to cervical dysplasia or cervical cancer. This is especially true in women younger than 30 years, in whom rates of HPV infection may be higher" (NCI, 2023).

Concerning co-testing, they recommend: "Based on solid evidence, screening every 5 years with the Pap test and the HPV DNA test (cotesting) in women aged 30 years and older is more sensitive in detecting cervical abnormalities, compared with the Pap test alone. Screening with the Pap test and HPV DNA test reduces the incidence of cervical cancer… Based on solid evidence, HPV and Pap cotesting is associated

with more false-positives than is the Pap test alone. Abnormal test results can lead to more frequent testing and invasive diagnostic procedures" (NCI, 2023).

Regarding screening women without a cervix, they recommend: "Based on solid evidence, screening is not helpful in women who do not have a cervix as a result of a hysterectomy for a benign condition" (NCI, 2023).

American Cancer Society (ACS)

The American Cancer Society updated their guidelines for cervical cancer screening for individuals at average risk in 2020. Their recommendations are summarized below:

(Adapted from Table 2 of (Fontham et al., 2020), *Comparison of Current and Previous American Cancer Society (ACS) Guidelines for Cervical Cancer Screening*)

Population	2020 ACS Recommendation
Age 21-24	No screening
Age 25-29	HPV test every 5 years (preferred) HPV/Pap cotest every 5 years (acceptable) Pap test every 3 years (acceptable)
Age 30-65	HPV test every 5 years (preferred) HPV/Pap cotest every 5 years (acceptable) Pap test every 3 years (acceptable)
Age 65 and older	No screening if a series of prior tests were normal

(Fontham et al., 2020).

American Society for Colposcopy and Cervical Pathology (ASCCP)

In 2019, the ASCCP published guidelines for cervical cancer screening in immunosuppressed women without an HIV infection. The following table was provided by Moscicki et al. (2019):

Table 3. Summary of Cervical Cancer Screening Recommendations for Non-HIV Immunocompromised Women

Risk group category	Recommendation
Solid organ transplant	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y

Cervical Cancer Screening, continued

	• If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If transplant before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age
Allogeneic hematopoietic stem cell transplant	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If transplant before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age • For HSCT patients who develop a new diagnosis of genital GVHD or chronic GVHD, resume annual cervical cytology until 3 consecutive normal results at which time perform cytology every 3 y, or perform an initial baseline co-test and, if cytology is normal and HPV is negative, perform co-testing every 3 y

Cervical Cancer Screening, continued

Inflammatory bowel disease on immunosuppressant treatments	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If on immunosuppressant therapy before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age
Inflammatory bowel disease not on immunosuppressant treatment	• Follow general population screening guidelines
Systemic lupus erythematosus and rheumatoid arthritis on immune-suppressant treatments	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If on immunosuppressant therapy before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age
Rheumatoid arthritis not on immunosuppressive treatments	• Follow general population screening guidelines
Type 1 diabetes mellitus	• Follow general population screening guidelines

Society of Gynecologic Oncology, American Society for Colposcopy and Cervical Pathology, American College of Obstetricians and Gynecologists, American Cancer Society, American Society of Cytopathology, College of American Pathologists, and the American Society for Clinical Pathology

Since the 2011 joint guidelines issued by the American Cancer Society, American Society for Colposcopy and Cervical Pathology , and American Society for Clinical Pathology Screening concerning cervical

cancer screening, additional reports regarding the use of primary hrHPV testing so that representatives from the Society of Gynecologic Oncology, American Society for Colposcopy and Cervical Pathology, American College of Obstetricians and Gynecologists, American Cancer Society, American Society of Cytopathology, College of American Pathologists, and the American Society for Clinical Pathology convened to issue interim clinical guidance in 2015. In the 2011 statement, primary hrHPV testing was not recommended. The 2015 recommendations include:

- "Because of equivalent or superior effectiveness, primary hrHPV screening can be considered as an alternative to current US cytology-based cervical cancer screening methods. Cytology alone and cotesting remain the screening options specifically recommended in major guidelines."
- "A negative hrHPV test provides greater reassurance of low CIN3+ risk than a negative cytology result."
- "Rescreening after a negative primary hrHPV screen should occur no sooner than every 3 years."
- "Primary hrHPV screening should not be initiated prior to 25 years of age."

Moreover, they give the following screening algorithm (Huh et al., 2015):

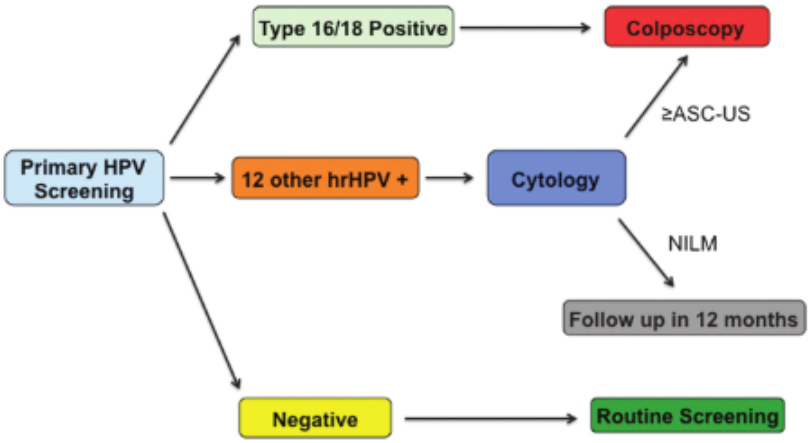

FIGURE 1. Recommended primary HPV screening algorithm. HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; NILM, negative for intraepithelial lesion or malignancy.

American College of Obstetricians and Gynecologists (ACOG)

In April 2021, the ACOG released a statement withdrawing and replacing the Practice Bulletin No.168 on cervical cancer screening, stating that it will be joining the ASCCP and the SGO "in endorsing the U.S. Preventive Services Task Force (USPSTF) cervical cancer screening recommendations, which replace ACOG Practice Bulletin No.168, *Cervical Cancer Screening and Prevention,* as well as the 2012 ASCCP cervical cancer screening guidelines. This was reaffirmed in 2023" (ACOG, 2021).

Cervical Cancer Screening, continued

In October 2020, the ACOG released "Updated Guidelines for Management of Cervical Cancer Screening Abnormalities." These consensus guidelines are based on risk to determine screening, surveillance, colposcopy, or treatment later in life (ACOG, 2020). In relation to screening, the updated management guidelines state:

1. "Recommendations are based on risk, not results.
    a. Recommendations of colposcopy, treatment, or surveillance will be based on a patient's risk of CIN 3+ determined by a combination of current results and past history (including unknown history). The same current test results may yield different management recommendations depending on the history of recent past test results.
2. Colposcopy can be deferred for certain patients.
    a. Repeat human papillomavirus (HPV) testing or cotesting at 1 year is recommended for patients with minor screening abnormalities indicating HPV infection with low risk of underlying CIN 3+ (e.g., HPV-positive, low-grade cytologic abnormalities after a documented negative screening HPV test or cotest).
3. All positive primary HPV screening tests, regardless of genotype, should have additional reflex triage testing performed from the same laboratory specimen (eg, reflex cytology).
    a. Additional testing from the same laboratory specimen is recommended because the findings may inform colposcopy practice. For example, those HPV-16 positive HSIL cytology qualify for expedited treatment.
    b. HPV 16 or 18 infections have the highest risk for CIN 3 and occult cancer, so additional evaluation (e.g., colposcopy with biopsy) is necessary even when cytology results are negative.
    c. If HPV 16 or 18 testing is positive, and additional laboratory testing of the same sample is not feasible, the patient should proceed directly to colposcopy.
4. Continued surveillance with HPV testing or cotesting at 3-year intervals for at least 25 years is recommended after treatment and initial posttreatment management of histologic HSIL, CIN 2, CIN 3, or AIS. Continued surveillance at 3-year intervals beyond 25 years is acceptable for as long as the patient's life expectancy and ability to be screened are not significantly compromised by serious health issues.
    a. New evidence indicates that risk remains elevated for at least 25 years, with no evidence that treated patients ever return to risk levels compatible with 5-year intervals.
5. Surveillance with cytology alone is acceptable only if testing with HPV or cotesting is not feasible. Cytology is less sensitive than HPV testing for detection of precancer and is therefore recommended more often. Cytology is recommended at 6-month intervals when HPV testing or cotesting is recommended annually. Cytology is recommended annually when 3-year intervals are recommended for HPV or cotesting.
6. Human papilloma virus assays that are Food and Drug Administration (FDA)-approved for screening should be used for management according to their regulatory approval in the United States. (Note: all HPV testing in [the guidelines] refers to testing for high-risk HPV types only).
    a. For all management indications, HPV mRNA and HPV DNA tests without FDA approval for primary screening alone should only be used as a cotest with cytology, unless sufficient,

rigorous data are available to support use of these particular tests in management" (ACOG, 2020).

American Society for Clinical Oncology (ASCO)

Resource-stratified recommendations were released in 2022 from the American Society for Clinical Oncology.

For maximal-based resource settings:

- "1.1. In maximal-resource settings, cervical cancer screening with HPV DNA testing should be offered every 5 years from age 25 to 65 years (either self- or clinician-collected). On an individual basis, women may elect to receive screening until age 70 years.
- 1.2. Women who are ≥ 65 years of age who have had consistently negative screening results during past ≥ 15 years may cease screening. Women who are 65 years of age and have a positive result after age 60 should be reinvited to undergo screening 2, 5, and 10 years after the last positive result. If women have received no or irregular screening, they should undergo screening once at 65 years of age, and if the result is negative, exit screening.
- 1.3. If the results of the HPV DNA test are positive, clinicians should then perform triage with reflex genotyping for HPV 16/18 (with or without HPV 45) and/or cytology as soon as HPV test results are known.
- 1.4. If triage results are abnormal (ie, ≥ ASC-US or positive for HPV 16/18 [with or without HPV 45]), women should be referred to colposcopy, during which biopsies of any acetowhite (or suggestive of cancer) areas should be taken, even if the acetowhite lesion might appear insignificant. If triage results are negative (e.g., primary HPV positive and cytology triage negative), then repeat HPV testing at the 12-month follow-up.
- 1.5. If HPV test results are positive at the repeat 12-month follow-up, refer women to colposcopy. If HPV test results are negative at the 12- and 24-month follow-up or negative at any consecutive HPV test 12 months apart, then women should return to routine screening
- 1.6. Women who have received HPV and cytology co-testing triage and have HPV-positive results and abnormal cytology should be referred for colposcopy and biopsy. If results are HPV positive and cytology normal, repeat co-testing at 12 months. If at repeat testing HPV is still positive, patients should be referred for colposcopy and biopsy, regardless of cytology results.
- 1.7. If the results of the biopsy indicate that women have precursor lesions (CIN2+), then clinicians should offer loop electrosurgical excision procedure (LEEP; if there is a high level of quality assurance [QA]) or, where LEEP is contraindicated, ablative treatments may be offered.
- 1.8. After women receive treatment for precursor lesions, follow-up should consist of HPV DNA testing at 12 months. If 12-month results are positive, continue annual screening; if not, return to routine screening" (ASCO, 2022).

In enhanced-resource settings:

Cervical Cancer Screening, continued

- "2.1. In enhanced-resource settings, cervical cancer screening with HPV DNA testing should be offered to women age 30-65 years, every 5 years (i.e., second screen 5 years from the first) (either self- or clinician-collected).
- 2.2. If there are two consecutive negative screening test results, subsequent screening should be extended to every 10 years.
- 2.3. Women who are ≥ 65 years of age who have had consistently negative screening results during past ≥ 15 years may cease screening. Women who are 65 years of age and have a positive result after age 60 should be reinvited to undergo screening 2, 5, and 10 years after the last positive result. If women have received no or irregular screening, they should undergo screening once at 65 years of age, and if the result is negative, exit screening.
- 2.4. If the results of the HPV DNA test are positive, clinicians should then perform triage with HPV genotyping for HPV 16/18 (with or without HPV 45) and/or reflex cytology.
- 2.5. If triage results are abnormal (ie, ≥ASC-US or positive for HPV 16/18 [with or without HPV 45]), women should be referred to colposcopy, during which biopsies of any acetowhite (or suggestive of cancer) areas should be taken, even if the acetowhite lesion might appear insignificant. If triage results are negative (e.g., primary HPV positive and cytology triage negative), then repeat HPV testing at the 12 month follow-up.
- 2.6. If HPV test results are positive at the repeat 12-month follow-up, refer women to colposcopy. If HPV test results are negative at the 12- and 24-month follow-up or negative at any consecutive HPV test 12 months apart, then women should return to routine screening.
- 2.7. If the results of colposcopy and biopsy indicate that women have precursor lesions (CIN2+), then clinicians should offer LEEP (if there is a high level of QA) or, where LEEP is contradicted, ablative treatments may be offered.
- 2.8. After women receive treatment for precursor lesions, follow-up should consist of HPV DNA testing at 12 months. If 12-month results are positive, continue annual screening; if not, return to routine screening" (ASCO, 2022).

In limited settings:

- "3.1. In limited settings, cervical cancer screening with HPV DNA testing should be offered to women 30 to 49 years of age every 10 years, corresponding to 2 to 3 times per lifetime (either self- or clinician-collected).
- 3.2. If the results of the HPV DNA test are positive, clinicians should then perform triage with reflex cytology (quality assured) and/or HPV genotyping for HPV 16/18 (with or without HPV 45) or with VIA. If institutions are currently using reflex cytology, they should transition from cytology to HPV genotyping.
- 3.3. If cytology triage results are abnormal (i.e. ≥ atypical squamous cells of undetermined significance [ASC-US]), women should be referred to quality assured colposcopy (the first choice, if available and accessible for women who are ineligible for thermal ablation), during which biopsies of any acetowhite (or suggestive of cancer) areas should be taken, even if the acetowhite lesion might appear insignificant. If colposcopy is not available, then perform VAT.

- 3.4. If HPV genotyping or VIA or VAT triage results are positive, then women should be treated. If the results from these forms of triage are negative, then repeat HPV testing at the 12-month follow-up.
- 3.5. If test results are positive at the repeat 12-month follow-up, then women should be treated.
- 3.6. For treatment, clinicians should offer ablation if the criteria are satisfied; if not and resources available, then offer LEEP.
- 3.7. After women receive treatment for precursor lesions, follow-up should consist of the same testing at 12 months" (ASCO, 2022).

Finally, in basic settings:

- "4.1. Health systems in basic settings should move to population-based screening with HPV testing at the earliest opportunity (either self- or clinician-collected). If HPV DNA testing for cervical cancer screening is not available, then VIA should be offered with the goal of developing health systems. Screening should be offered to women 30 to 49 years of age, at least every 10 years (increasing the frequency to every 5 years, resources permitting).
- 4.2. If the results of available HPV testing are positive, clinicians should then perform VAT followed by treatment with thermal ablation and/or LEEP, depending on the size and location of the lesion.
- 4.3. If primary screening is VIA and results are positive, then treatment should be offered with thermal ablation and/or LEEP, depending on the size and location of the lesion.

  4.4. After women receive treatment for precursor lesions, then follow up with the available test at 12 months. If the result is negative, then women return to routine screening" (ASCO, 2022).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA has approved the APTIMA HPV 16 18/45 Genotype Assay, a nucleic acid amplification test (NAAT), for the qualitative detection of mRNA for HPV 16, 18, and 45 from Gen-Probe Incorporated on October 12, 2012; however, this test cannot distinguish between 18 and 45. Previously, on October 28, 2011, the FDA approved Gen-Probe Incorporated's APTIMA HPV Assay, an NAAT that tests for 14 high-risk types of HPV but is unable to distinguish between the 14 types.

Hologic, Inc. has two FDA-approved HPV NAAT tests—Cervista HPV 16/18 and Cervista HPV HR and GENFIND DNA Extraction Kit. Both were approved on March 12, 2009. The former is a fluorescent, isothermal-based reaction that detects HPV 16 and 18 whereas the latter screens for DNA from the 14 high-risk HPV strains (FDA, 2023a).

The COBAS HPV test by Roche Molecular Systems, Inc. was approved by the FDA on April 19, 2011, as a NAAT for 14 high-risk types of HPV. This test can specifically identify HPV 16 and 18 but cannot distinguish from the other 12 types of HPV. On July 2, 2018, the FDA released an approval order statement (P100020/S025) "for an expansion of the intended use for the FDA-approved cobas HPV Test to include cervical specimens collected in SurePath Preservative Fluid as a specimen type" (FDA, 2023c). This approval allows for the cobas HPV Test to be used as a first-line cervical cancer screening using the

SurePath preservative, a medium often used for Pap tests (Rice, 2018). In 2020, the Cobas HPV was FDA approved for use on Cobas 6800/8800 Systems (FDA, 2023b).

On February 12, 2018, the FDA approved the BD Onclarity™ HPV Assay which detects 14 high-risk HPV genotypes including high-risk strains 16 and 18. "The BD Onclarity HPV Assay is a qualitative in vitro test for the detection of Human Papillomavirus in cervical specimens collected by a clinician using an endocervical brush/spatula combination or broom and placed in BD SurePath vial" (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.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
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
88141	Cytopathology, cervical or vaginal (any reporting system), requiring interpretation by physician
88142	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; manual screening under physician supervision
88143	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; with manual screening and rescreening under physician supervision
88147	Cytopathology smears, cervical or vaginal; screening by automated system under physician supervision
88148	Cytopathology smears, cervical or vaginal; screening by automated system with manual rescreening under physician supervision
88150	Cytopathology, slides, cervical or vaginal; manual screening under physician supervision
88152	Cytopathology, slides, cervical or vaginal; with manual screening and computer-assisted rescreening under physician supervision
88153	Cytopathology, slides, cervical or vaginal; with manual screening and rescreening under physician supervision
88164	Cytopathology, slides, cervical or vaginal (The Bethesda System); manual screening under physician supervision
88165	Cytopathology, slides, cervical or vaginal (The Bethesda System); with manual screening and rescreening under physician supervision

Cervical Cancer Screening, continued

88166	Cytopathology, slides, cervical or vaginal (The Bethesda System); with manual screening and computer-assisted rescreening under physician supervision
88167	Cytopathology, slides, cervical or vaginal (The Bethesda System); with manual screening and computer-assisted rescreening using cell selection and review under physician supervision
88174	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; screening by automated system, under physician supervision
88175	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; with screening by automated system and manual rescreening or review, under physician supervision
0500T	Infectious agent detection by nucleic acid (DNA or RNA), Human Papillomavirus (HPV) for five or more separately reported high-risk HPV types (e.g., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) (i.e., genotyping)
G0123	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, screening by cytotechnologist under physician supervision
G0124	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, requiring interpretation by physician
G0141	Screening cytopathology smears, cervical or vaginal, performed by automated system, with manual rescreening, requiring interpretation by physician
G0143	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with manual screening and rescreening by cytotechnologist under physician supervision
G0144	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with screening by automated system, under physician supervision
G0145	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with screening by automated system and manual rescreening under physician supervision
G0147	Screening cytopathology smears, cervical or vaginal, performed by automated system under physician supervision
G0148	Screening cytopathology smears, cervical or vaginal, performed by automated system with manual rescreening
G0476	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) for cervical cancer screening, must be performed in addition to pap test
P3000	Screening Papanicolaou smear, cervical or vaginal, up to three smears, by technician under physician supervision
P3001	Screening Papanicolaou smear, cervical or vaginal, up to three smears, requiring interpretation by physician
Q0091	Screening Papanicolaou smear; obtaining, preparing and conveyance of cervical or vaginal smear to 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
February 13, 2023	Modified wording in coverage criteria to reflect 'individual(s)' instead of 'women': "Within these coverage criteria, "individual(s)" is specific to individuals with a cervix." Also, modified wording and formatting for overall coverage criteria.
January 8, 2024	The following changes were implemented: Removed previous coverage criteria #1; modified new coverage criteria #1 (previously coverage criteria #2) to allow for screening in immunocompromised individuals of all ages: a) defines under age 30, b) defines over age 30; switched the order of coverage criteria #4 and #5 such that testing for high-risk individuals over age 65 immediately follows frequency testing for those ages 30 to 65 (coverage criteria #4 ha now been edited for clarity on frequency testing allowed in high-risk individuals over age 65); addition of new coverage criteria #7: "For all situations not addressed above, cervical cancer screening (cervical cytology, HPV testing) for individuals less than 21 years of age **DOES NOT MEET COVERAGE CRITERIA.**"

Cervical Cancer Screening, continued

G2002 Cervical Cancer Screening

Coronavirus Testing in the Outpatient Setting

Policy #: AHS – G2174	**Prior Policy Name & Number (as applicable):**
Implementation Date: 12/1/23	**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

Human coronaviruses, first characterized in the 1960s, are named based on the spiked proteins located on their surface. As of 2020, seven coronaviruses are known to infect humans. Four, of which—229E, NL63, OC43, and HKU1—are associated with the common cold. MERS-CoV is the coronavirus that causes Middle East Respiratory Syndrome, or MERS. SARS-CoV is the causative agent of Severe Acute Respiratory Syndrome (SARS), and SARS-CoV-2 is the virus that causes coronavirus disease 2019, or COVID-19 (CDC, 2020b). As of November 11, 2022, the United States had reported more than 634,709,332 confirmed cases of COVID-19 and over 6,609,119 reported COVID-19 deaths (JHU, 2022). Testing for a possible coronavirus infection can include molecular tests, such as nucleic acid-based testing like reverse transcription polymerase chain reaction (RT-PCR); host antibody testing; and antigen testing.

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. Targeted nucleic acid testing, such as RT-PCR, for COVID-19 (SARS-CoV-2), including rapid molecular tests, **MEETS COVERAGE CRITERIA** in the following situations:
   a) For individuals displaying signs and symptoms of possible COVID-19 infection (See Note 1).

b) For asymptomatic individuals with known exposure to COVID-19, EXCEPT when the individual has had a previous COVID-19 infection within the last 90 days.

c) For asymptomatic individuals prior to undergoing immunosuppressive or aerosol-producing procedures

d) For individuals with signs or symptoms of SARS who have traveled to endemic areas or who have been exposed to persons with SARS, targeted nucleic acid testing, such as RT-PCR, for the detection of severe acute respiratory syndrome (SARS) coronavirus RNA **MEETS COVERAGE CRITERIA**.

2. For individuals with signs or symptoms of Middle East respiratory syndrome (MERS) who have traveled to endemic areas or who have been exposed to persons with MERS, targeted nucleic acid testing, such as RT-PCR, for the detection of MERS coronavirus RNA **MEETS COVERAGE CRITERIA**.

3. Host antibody serology testing to support a diagnosis of multisystem inflammatory syndrome in children (MIS-C) (see Note 2), multisystem inflammatory syndrome in adults (MIS-A) (see Note 3), **or** post-acute sequelae of SARS-CoV-2 infection (PASC) **MEETS COVERAGE CRITERIA**.

4. For symptomatic individuals, the use of an antigen-detecting diagnostic test for SARS-CoV-2, including antigen rapid tests, **MEETS COVERAGE CRITERIA**.

5. For individuals with signs and symptoms of a respiratory tract infection (see Note 4), multiplex PCR-based panel testing of up to **5** respiratory pathogens **MEETS COVERAGE CRITERIA**.

6. For individuals with signs and symptoms of a respiratory tract infection (see Note 4), antigen panel testing of up to **5** antigens **MEETS COVERAGE CRITERIA**.

7. For the diagnosis of SARS-CoV-2 reinfection, whole genome sequencing of paired specimens from distinct lineages (as defined in Nextstrain or GISAID) **DOES NOT MEET COVERAGE CRITERIA**.

8. Antigen panel testing of **6 or more** antigens **DOES NOT MEET COVERAGE CRITERIA**.

9. Multiplex PCR-based panel testing of **6 or more** respiratory pathogens **DOES NOT MEET COVERAGE CRITERIA**.

10. For all other situations not described above, host antibody serology 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.

11. For all situations, neutralization antibody testing for SARS-CoV-2 **DOES NOT MEET COVERAGE CRITERIA.**

12. Testing for other endemic coronaviruses, such as 229E, NL63, OC43, and HKU1, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Signs and symptoms associated with a possible COVID-19 infection can include a fever, cough, fatigue, shortness of breath or difficulty breathing, congestion or runny nose, chills, muscle pain, headache, sore throat, new loss of taste or smell, nausea, vomiting, diarrhea, conjunctivitis, rash on skin or discoloration of fingers or toes (CDC, 2020f; WHO, 2020d).

Note 2: According to the CDC, evidence of possible MIS-C includes (CDC, 2021d):

- Fever of at least 38.0˚C for at least 24 hours
- Multisystem (2 or more) organ involvement
- Laboratory evidence of inflammation, "including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin (CDC, 2020d)"

Some children may fulfill full or partial criteria for Kawasaki disease

Note 3: According to the CDC, evidence of possible MIS-A includes (Morris et al., 2020; Patel et al., 2021):

- a severe illness requiring hospitalization in a person aged ≥ 21 years;
- a positive test result for current or previous SARS-CoV-2 infection (nucleic acid, antigen, or antibody) during admission or in the previous 12 weeks;
- severe dysfunction of one or more extrapulmonary organ systems (e.g., hypotension or shock, cardiac dysfunction, arterial or venous thrombosis or thromboembolism, or acute liver injury);
- laboratory evidence of severe inflammation (e.g., elevated CRP, ferritin, D-dimer, or interleukin-6);
- absence of severe respiratory illness (to exclude patients in which inflammation and organ dysfunction might be attributable simply to tissue hypoxia).

Note 4: Signs and symptoms of a respiratory tract infection:

- A temperature greater than 102°F
- Pronounced dyspnea,
- Tachypnea, or
- Tachycardia.

III. Scientific Background

On March 11, 2020, the World Health Organization (WHO) declared the novel coronavirus SARS-CoV-2, or COVID-19, a global pandemic (Cucinotta & Vanelli, 2020). COVID-19 is the third recent human coronavirus to be declared an emergency. SARS (Severe Acute Respiratory Syndrome) was recognized as an emergency by the WHO in February 2003 (WHO, 2022c). This outbreak in 2003 resulted in over 8000 cases in 26 different countries. Since 2003, only four limited reoccurrences have been reported according to the WHO—three incidences are due to laboratory accidents (in Taipei and Singapore) and one incident of undetermined source in China (WHO, 2022c). As early as September 2012, another

human coronavirus, MERS-CoV, began to spread in the Middle East, causing Middle East Respiratory Syndrome (MERS). Although the WHO did not initially declare MERS an emergency, they have since added MERS to their list of pandemic/epidemic diseases. Since September 2012 and as of the end of October 2021, the WHO reports 2574 laboratory-confirmed cases of MERS with 858 MERS-associated deaths (34.4% fatality rate) in 27 countries (WHO, 2022b).

Unlike the initial SARS and MERS outbreaks that were predominantly regionally contained, COVID-19 became a global pandemic. According to the WHO, as of November 11,2022, there were more than 630 million confirmed cases of COVID-19 with over 6,584,104 confirmed deaths worldwide (WHO, 2022a). Infection from the novel human coronavirus SARS-CoV-2 can result in coronavirus disease 2019 (COVID-19). The WHO reports approximately 15% of individuals with COVID-19 develop severe disease requiring oxygen support while 5% develop "critical disease" with complications such as respiratory failure or multiorgan failure (WHO, 2021b). Older individuals and patients with comorbidities—such as cardiovascular disease, diabetes mellitus, hypertension, chronic lung disease, cancer, chronic kidney disease, obesity, and smoking—have an increased likelihood of poor outcomes (McIntosh, 2021). Sepsis, multiorgan failure (including the kidney, liver, and heart), pneumonia, and acute respiratory distress syndrome (ARDS) can also occur (WHO, 2021b; Yang et al., 2020). Severe outcomes have been associated with the following laboratory features: lymphopenia, elevated liver enzymes, elevated lactate dehydrogenase (LDH), elevated inflammatory markers (such as CRP and ferritin), elevated D-dimer, elevated prothrombin time (PT), elevated troponin, elevated creatine phosphokinase (CPK), and acute kidney injury (McIntosh, 2021).

Much of what has generated this global pandemic is attributed to the different levels of transmissibility of the SARS-CoV-2 virus compared to SARS-CoV-1 and MERS, which can arise from the viral load. Simply put, viral load is the number of viral particles/virions in a milliliter of blood (Ryding, 2020). The viral load of SARS-CoV-2 "peaks around the time of symptom onset, followed by a gradual decrease to a low level after about 10 days. Regarding the period of high infectiousness, a recent study reported that exposure to an index case within 5 days of symptom onset confers a high risk of secondary transmission" (Kawasuji et al., 2020). This finding was corroborated by other studies, which found that "SARS-CoV-2 viral load in the upper respiratory tract appeared to peak in the first week of illness, whereas that of SARS-CoV peaked at days 10–14 and that of MERS-CoV peaked at days 7–10;" because SARS-CoV-2 viral load peaks faster, it can be more transmissible earlier in the disease course (Cevik et al., 2021). However, after reaching its peak during symptom onset, the viral load decreases "monotonically" (Kawasuji et al., 2020). If viral loads do not decrease, patients will be more likely to suffer worse outcomes and require hospitalization (Griffin, 2020). Viral load has been found to be either similar among symptomatic and asymptomatic COVID-19 positive individuals, or higher among symptomatic individuals (Kawasuji et al., 2020; Nackerdien, 2020). Infectiousness of COVID-19 also correlates with shedding, meaning that the viral particles can replicate in an individual and spread in the environment to others. The mean duration of SARS-CoV-2 RNA shedding "was 17.0 days (95% CI 15·5–18·6; 43 studies, 3229 individuals) in upper respiratory tract, 14.6 days (9·3–20·0; seven studies, 260 individuals) in lower respiratory tract, 17.2 days (14·4–20·1; 13 studies, 586 individuals) in stool, and 16.6 days (3·6–29·7; two studies, 108 individuals) in serum samples," with maximum shedding duration reaching "83 days in the upper respiratory tract, 59 days in the lower respiratory tract, 126 days in stools, and 60 days in serum"(Cevik et al., 2021).

In children and adolescents, reports of a multisystem inflammatory syndrome (MIS-C) with similarities

to Kawasaki disease and toxic shock syndrome have been linked to COVID-19 (CDC, 2020d; DeBiasi et al., 2020; Jones et al., 2020; Verdoni et al., 2020; WHO, 2020c). Multisystem inflammatory syndrome has also been reported in adults (MIS-A). From June to October 2020, researchers reported 27 cases of MIS-A in the US and UK (Baum, 2020). The case definition of MIS-A includes "(1) hospitalization without evidence of severe respiratory illness (to exclude hypoxia as the cause of the signs and symptoms), (2) extrapulmonary organ system involvement (including hypotension or shock, cardiac dysfunction, arterial or venous thromboembolism, acute liver injury, or dermatologic abnormalities), and (3) laboratory evidence of acute inflammation (e.g., highly elevated C-reactive protein, ferritin, D-dimer, or interleukin-6)" (Baum, 2020). Most patients present with a fever > 100.4 °F, cardiac abnormalities (arrhythmias, elevated troponin levels, or left or right ventricular dysfunction), and gastrointestinal symptoms. Rare symptoms include dermatological manifestations or respiratory symptoms such as pleural effusion. Patients may have elevated laboratory markers of inflammation including CRP, ferritin, and markers of coagulopathy including D-dimer (Morris et al., 2020).

On September 20, 2020, the first case of a new COVID-19 variant, B.1.1.7 (Alpha) or VUI-202012/01 (VUI for "variant under investigation") was recorded in the UK. As of August 11, 2021, the Beta and Gamma variants are not classified as "variants of concern" (CDC, 2022r). The Gamma variant is now considered a "variant being monitored" or VBM (CDC, 2022c).

The B.1.617.2 (Delta) variant was first detected in the US in March 2021, but had its first case in India in December 2020 (CDC, 2022r). The delta variant is classified as a "variant of concern." Individuals with the delta variant also have viral loads "up to 1,260 times higher" than compared to those infected by the original virus, and as of July 2021, has been detected in more than 130 countries (GVN, 2021). It is also more contagious than other illnesses such as "MERS, SARS, Ebola, the common cold, the seasonal flu, and smallpox, and it is as contagious as chickenpox" (Mandavilli, 2021). It has been associated with different symptoms than other strains; "fever, headache, sore throat, and runny nose are common, while cough and loss of smell are not. Other reports link Delta to more serious symptoms, including hearing impairment, severe gastrointestinal issues and blood clots leading to tissue death and gangrene" (ASM, 2021). However, between August 7, 2022 and October 22, 2022, the Delta variant accounted for 0.0% of COVID-19 cases; Omicron accounted for the majority of cases (CDC, 2022c).

B.1.1.529 (Omicron) variant was first reported to WHO in South Africa on November 24, 2021 and on December 1, 2021, the first case of Omicron was confirmed in the US (CDC, 2022l). Omicron variant is comprised of four different lineages, which include BA 1.1, B.1.1.529, BA.2, BA.2.12.1, and BA.3 (CDC, 2022k, 2022p). The Omicron variant was classified as a "variant of concern" (CDC, 2022c). At first, Omicron variant was detected in travel-related cases in several European countries, as well as Australia, Brazil, Canada, Hong Kong, Israel, Japan, Nigeria, Norway, Sweden, and the United Kingdom. However, cases doubled every 2-3 days in the UK thereafter and rose from 1% to 99% of infections nationally in 6 weeks, compared with 18 weeks for Delta. Omicron may be over 10 times more contagious than the original virus or about 2.8 times as infectious as the Delta variant due to mutations in the spike protein (CDC, 2022f). Symptoms are similar to previous variants but has shown to cause less severe disease which depends on COVID-19 vaccination status, the presence of other health conditions, age, and history of prior infection (CDC, 2022l). According to Our World in Data, by November 11, 2022, 68.2% of the US population had received at least one dose of a COVID-19 vaccine (Mathieu et al., 2022).

The CDC has listed the Pfizer-BioNTech COVID-19 Vaccine, Moderna COVID-19 Vaccine, and Johnson & Johnson's (J&J) Janssen COVID-19 Vaccine as the only three authorized and recommended vaccines to prevent COVID-19 in the US (CDC, 2022d). The Pfizer-BioNTech and Moderna COVID-19 vaccines are mRNA vaccines, which instruct B and T lymphocytes to fight off that specific mRNA-encoded protein from COVID-19 in the event of future exposure. They both require two doses to obtain optimal effectiveness (CDC, 2022o). After the two doses, the Pfizer vaccine and Moderna vaccine are 95% and 94.1% effective, respectively, in preventing COVID-19 (Branswell, 2020). The Johnson & Johnson/Janssen COVID-19 vaccine is a viral vector vaccine, which uses a "modified version of a different virus (the vector) to deliver important instructions to our cells" (CDC, 2022q). The Janssen COVID-19 vaccine only requires one dose, and in clinical trials, the vaccine was 66.3% effective at "preventing laboratory-confirmed COVID-19 illness in people who had no evidence of prior infection 2 weeks after receiving the vaccine" (CDC, 2021c). For primary and booster vaccination, CDC notes a preference for mRNA vaccines (Pfizer-BioNTech or Moderna) over the Janssen COVID-19 Vaccine. However, the Janssen vaccine is preferable in instances where there are no other vaccines available (CDC, 2022d).

As of August 31, 2022, there are 42 vaccines in Phase 3 trials and 10 in combination phase 2/3 trials (Zimmer et al., 2022). AstraZeneca and Novavax are approved for usage in the EU (AstraZeneca, 2021; Zimmer et al., 2022). AstraZeneca is a vector vaccine, which contains weakened versions of a virus that has the same entryway as COVID-19 into a cell, and Novavax is a protein subunit vaccine, which contains stabilized but immunogenic pieces of the SARS-CoV-2 virus, namely the spike protein (AstraZeneca, 2021; CDC, 2022o; NIH, 2020). AstraZeneca and Novavax COVID-19 vaccines require two doses (AstraZeneca, 2021; Roberts, 2021). The AstraZeneca and Novavax COVID-19 vaccines have been found to be up to 90% effective in UK trials (Roberts, 2021; Van Beusekom, 2020).

With the emergency of the Omicron variant in November 2021, further research suggested that vaccine effectiveness at preventing infection wanes over time, especially in those aged 65 years and older. Data from clinical trials showed that a booster shot increased the immune response in trial participants who finished a Pfizer-BioNTech or Moderna primary series 6 months earlier or who received a J&J/Janssen single-dose vaccine 2 months earlier. For Pfizer-BioNTech and J&J/Janssen, clinical trials also showed that a booster shot helped prevent severe symptoms of COVID-19 (CDC, 2022b).

Vaccines in clinical trials have focused on targeting the spike (S) protein (based on experience with SARS-CoV-1), which contains a receptor binding domain (RBD) responsible for allowing entrance into host cells and is the current target for neutralizing antibodies. Other targets could potentially include the nucleocapsid (N) protein, likely for inactivated virus or live attenuated approaches, or T cell epitopes, which may provide additional protection. Knowing that T-cell responses against structural proteins in SARS-CoV-1 were more immunogenic than non-structural proteins could be key in manufacturing more effective vaccines for SARS-CoV-2 as well (Tregoning et al., 2020).

Besides the viruses associated with SARS, MERS, and COVID-19, four other human coronaviruses (HCoVs) are currently known—229E, NL63, OC43, and HKU1. These four viruses are considered endemic to the human population, and they typically cause mild respiratory tract infections associated with the common cold; in fact, it is approximated that up to one-third of all "common colds" may be due to one of these four endemic human coronaviruses. These HCoVs can cause both upper and lower respiratory infections, but they typically result in relatively mild, or even asymptomatic, cases. In

immunosuppressed individuals, including those with pre-existing pulmonary diseases, progression to acute respiratory failure can occur in some cases (Corman et al., 2019; Ludwig & Zarbock, 2020).

Nucleic Acid Testing for Human Coronavirus Infections

Coronaviruses are a family of enveloped, single-stranded positive-sense RNA viruses. During the initial phase of infection, the virus can be detected in respiratory specimen due to high concentrations of viral RNA **(Figure 1).** RT-PCR is a powerful molecular technique that synthesizes complimentary DNA (cDNA) from the initial RNA template and uses primers to manufacture multiple cDNA copies for analysis. RT-PCR, when used with appropriate primers targeting the SARS-CoV-2 RNA, is used to diagnose an acute infection. The CDC RT-PCR Diagnostic Panel detects SARS-CoV-2 virus in the upper and lower respiratory specimen. The CDC has released standard primers to detect SARS-CoV-2 RNA, but any primers or probes that receive an Emergency Use Authorization (EUA) label may also be used with the CDC's RT-PCR Diagnostic Panel (CDC, 2020e). As depicted in **Figure 1**, the concentration of viral RNA decreases as the immune system fights the infection, and very low or undetectable viral RNA levels are typically present after an individual has recovered. Consequently, RT-PCR cannot be used to screen for a past infection. Another limitation to RT-PCR is that it does require specific instrumentation, and, therefore, is less amenable as a rapid, point-of-care test. RT-PCR results of SARS-CoV-2 may fluctuate and become unstable over time, thus requiring other clinical diagnostic measures, such as computerized tomography (CT imaging) to supplement isolation, discharge, and any transfers during this epi demic (Li et al., 2020).

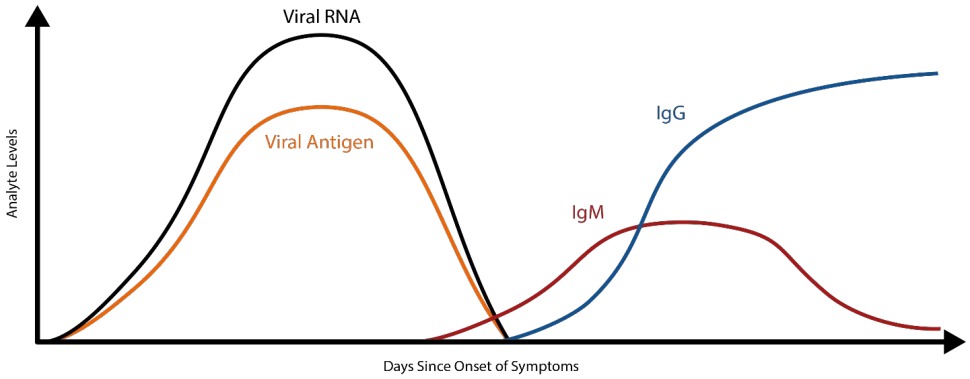

Figure 1: *General time course of a viral infection, such as SARS-CoV-2. This is for illustrative purposes and should not be used as a primary reference or for diagnostic purposes. The original content can be found within the references (The_Native_Antigen_Company, 2020).*

Clinical Utility and Validity of Nucleic Acid Testing

Many studies have been performed to date to evaluate the analytical performance of RT-PCR. One study, using a high-throughput platform, for example, reported a limit of detection (LoD) of 689.3 copies/mL and 275.72 copies per reaction at 95% detection probability (Pfefferle et al., 2020). The WHO diagnostic

RT-PCR test utilizes two genes--the *E* gene as the molecular target (where the limit is 3.9 copies per reaction) and the *RdRp* gene as the molecular target (limit of 3.6 copies per reaction) (Lippi et al., 2020). One recent study reported possible *in vitro* cross-reactivity between the RdRp-based method used predominantly in European labs with SARS-CoV in cell culture (Chan et al., 2020). SARS-CoV is the coronavirus that caused the initial SARS (Severe Acute Respiratory Syndrome) outbreak in 2003 (WHO, 2022c). The likelihood of either a co-infection of SARS-CoV and SARS-CoV-2 or a concurrent outbreak of both viruses is small. The CDC diagnostic panel test does not target the *RdRp* gene; it consists of two primer/probe sets of the *N* gene and one primer/probe set for human RNase P gene (*RP*) as the control. The CDC diagnostic panel has a reported limit of 1.0 – 3.2 copies/µL (CDC, 2020a; Lippi et al., 2020). Reports of initial negative RT-PCR results in individuals who later develop symptomatic COVID-19 have been published, but this may occur if the sample was not properly collected or if it was taken from the patient early in the infection during the initial incubation period of SARS-CoV-2, which is approximately 6 days (interquartile range [IQR], 2 – 11 days) (Backer et al., 2020; Lippi et al., 2020). Consequently, it is important to remember that "Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information (LabCorp, 2020b)."

To compare and analyze the diagnostic efficacy of two RT-PCR test kits for detection of SARS-CoV-2, Lu et al. (2020) studied throat swab samples from 18 hospitalized patients with a clinical COVID-19 diagnosis and 100 hospitalized patients without COVID-19 diagnosis. Two different RT-PCR tests from Sansure Biotech Inc (SansureBiotech, 2020) and Shanghai BioGerm Biotechnology Co., Ltd (BioGerm, 2020) were used. The table below (Lu et al., 2020) shows that the detection efficacy of BioGerm PCR kit was higher than that of the Sansure PCR kit. These two kits had the same specificity and positive predictive value, but the sensitivity of the Sansure PCR kit was 83.3%, whereas the sensitivity of the BioGerm PCR kit was 94.4%. For the Sansure PCR kit, 3 of the 18 samples were false-negative results, and for the BioGerm PCR kit, 1 of the 18 samples was a false-negative result. No false-positive results were detected in these tests. The author suggests that "these findings provide important information for the ongoing optimization of viral detection assays following the emergence of COVID-19" (Lu et al., 2020).

	COVID-19 samples (n = 18)		None- COVID-19 samples (n = 100)		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Kappa (95%CI)
Test kits	Positive	Negative	Positive	Negative					
Sansure	15	3	0	100	0.833(0.577-0.956)	1.000(0.954-1.000)	1.000(0.747-1.000)	0.971(0.911-0.992)	0.894(0.726-1.000)
BioGerm	17	1	0	100	0.944(0.706-0.997)	1.000(0.954-1.000)	1.000(0.771-1.000)	0.990(0.938-0.999)	0.966(0.880-1.000)

Table 2. *Diagnosis efficacy of Sansure and BioGerm test kits for SARS-CoV-2 nucleic acid detection*

In a case series study of multisystem inflammatory syndrome in adults (MIS-A) associated with SARS-CoV-2 infection, 16 patients ranging from 21 to 50 years old were enrolled and tested with PCR assay. 10 out of 16 patients had positive SARS-CoV-2 PCR test results at the time of admission. Two patients had positive SARS-CoV-2 PCR test results 14 and 37 days before admission and negative PCR results at the time of admission. Three patients had positive SARS-CoV-2 PCR test results 25–41 days before admission and continued positive PCR test results at the time of admission. “Given the high proportion of MIS-C patients with negative PCR testing, clinical guidelines recommend the use of both antibody and viral testing to assist with diagnosis” (Morris et al., 2020).

Li et al. (2021) conducted a cross-sectional analysis on 30 patients with COVID-19 diagnoses to compare the sensitivity of SARS-CoV-2 testing in anterior nasal vestibular swabs versus oropharyngeal swabs. After specimen collection, RT-PCR assays were used to test them for SARS-CoV-2. They found that 56.7% of the patients tested positive using oropharyngeal specimen, whereas 66.7% of patients tested positive with the nasal swab specimens. Ultimately, there is “adequate sensitivity” to use the less invasive anterior nasal vestibular swabs to detect COVID-19 infection confirmed by RT-PCR (Li et al., 2021).

Yau et al. (2021) evaluated the clinical utility of a rapid “on-demand” PCR-based testing service in an acute hospital setting. In an effort to increase hospital efficiency starting from July 2020, the researchers focused on moving patients quickly to isolation rooms and minimize potential risk of transmission in crowded areas. From their study, it was found that the “daily/monthly PCR positive test numbers approximately followed the local and national UK trend in COVID-19 case numbers, with the daily case numbers being reflective of the Nov and Dec 2020 surges.” It ultimately helped to reduce “unnecessary ‘length-of-stay’ in a busy acute respiratory ward.” Patients were able to be rapidly separated based on COVID-19 positive diagnosis and the system in place reduced exposure and nosocomial transmission (Yau et al., 2021).

Dighe studied a lateral flow strip-based RNA extraction and amplification-free nucleic acid test (NAT) for rapid diagnosis of COVID-19 at point of care which takes no longer than 30 minutes. This test uses highly specific 6-carboxyfluorescein (6-FAM) and biotin labeled antisense oligonucleotides (ASOs) as probes those are designed to target the N-gene sequence of COVID-19. This study evaluated 60 samples using the lateral flow assay and results were compared with the FDA-approved TaqPath RT-PCR kit. According to the results, the assay obtained almost 99.99% accuracy and specificity. The authors conclude that this new LFA method could be "expanded beyond COVID-19 detection, simply by altering its targeting antisense oligonucleotides, to become a global health technology that contributes to providing low-cost diagnostics" (Dighe et al., 2022).

Mawhorter et al. (2022) investigated the impact and cost of a routine pre-operative COVID-19 PCR testing algorithm for asymptomatic patients before elective surgery at a rural academic institution per recommendations by the American College of Surgeons. From 7579 pre-procedural tests that were completed since May 2020 using the protocol, the study yielded 31 (0.41%) positive results in asymptomatic patients. With these positive results, there were impacts on both the cost and delay of the procedure. The results showed that “20 procedures (62.5%) were delayed an average of 49 days, 8 were not performed, and 3 proceeded without delay,” with a prolonged delay for the 3 urological procedures of 59 days. They also identified that the number needed to test for one positive result was 244, with $11,573 as cost for each positive result. This analysis found that the hospital was able to be more cost-effective (each test was $34-54) with a standardized testing algorithm prior to procedure performance (Mawhorter et al., 2022).

Host Antibody Testing

The COVID-19 illness begins with an initial infection by SARS-CoV-2. Viral invasion stimulates the host immune response to produce immunoglobulins, such as IgM, IgA, and IgG, that can target the invading virus. However, there is a delay between the time of initial infection and the production of immunoglobulins **(Figure 1)** (The_Native_Antigen_Company, 2020). Typically, several days after the initial onset of symptoms, the first IgM immunoglobulins are produced to combat the viral infection. IgA (not shown in Figure 1), immunoglobulins secreted to protect predominantly the mucosal linings of the gastrointestinal, respiratory, and genitourinary tracts (Woof & Kerr, 2006), typically have a half-life of 4 – 6 days (Morell et al., 1973). Finally, IgG, the long-term immunoglobulins found within body fluids that fight bacterial and viral infections, are produced and IgM production wanes. Some limited studies have indicated that some individuals may initially produce IgM and IgG antibodies concurrently, but additional research is needed (Padoan et al., 2020).

Serological host antibody tests can detect the presence of IgM and IgG antibodies that an individual has developed in response to an infection—in this case, a SARS-CoV-2 viral infection. The test may report total antibodies present, meaning either it does not distinguish between IgG and IgM or that it is reporting the sum of IgG and IgM. This is sometimes referred to as “total antibody testing”. On the other hand, the test may be specific for one antibody, such as IgG or IgM, or the test may claim to accurately distinguish between the antibodies.

Another type of antibody testing is “neutralizing” antibody detection, as opposed to “binding” antibody detection described above. This process involves incubating serum with a live version of the virus. The

analytes of interest are the antibodies that have the capability to prevent infection by the virus (i.e. neutralization). Identification of these antibodies may contain useful clinical information and are often reported in an aggregate titer, as opposed to specifying each individual antibody (CDC, 2022h; Espejo et al., 2020). Due to use of live virus, these tests may need to be performed in a higher biosafety laboratory, although some techniques using pseudoviruses may be performed in less restrictive laboratories (CDC, 2022i).

Clinical Utility and Validity of Host Antibody Testing

Antibody testing has many potential uses. Ideally, the use of an accurate, reliable antibody test could possibly show whether someone has previously been exposed to the virus. This could indicate possible immunity in an individual. Please note that ***the antibody test is not used as a diagnostic test, meaning it should not be used to diagnose an acute infection***. Within the FDA policy for diagnostic testing for COVID-19, issued on November 15, 2021 they state, "Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infection" (FDA, 2022e).

The FDA published a Frequently Asked Questions (FAQ) update on March 19, 2021. In it, they remarked that antibody tests cannot be used to diagnose COVID-19, and that molecular or antigen tests are available for diagnostic purposes (FDA, 2022a).

Since SARS-CoV-2 is a new, emerging virus, it is not known for certain how long it takes for the seroconversion to occur or when antibodies start to appear in the blood at high enough concentrations for accurate testing results. A recent study published in *Clinical Infectious Diseases* reports an average of seroconversion time for IgM and IgG at 12 and 14 days, respectively (Zhao et al., 2020). A small study (n=34 patients) reports the presence of IgG for at least seven weeks (the duration of the study) (Xiao et al., 2020). Another study, however, reports that IgM testing has similar, if not better positive detection rate than PCR 5.5 days after initial onset of symptoms; however, the total window of antibody detection for IgM was only 5 days long (Guo et al., 2020) **(See Figure 1)**. If the patient was not tested during the detection window, then the individual would not necessarily have a "positive" result for IgM. The authors also report the detection of IgA antibodies (median onset at 5 days after initial symptoms [IQR 3 – 6 days]), and 92.7% of total samples report a positive result for IgA. This same study also reports that IgG detection occurs, on average, fourteen days after initial onset of symptoms (Guo et al., 2020). Another study reports that IgA-based ELISA testing has higher sensitivity than IgG-based ELISA testing, but the IgG-based ELISA testing has higher specificity. The authors recommend IgG-based testing over the IgA-based testing in immunosurveillance studies since IgG has a longer biological half-life (Okba et al., 2020). At least one published study to date has reported that as many as 6.9% of individuals who previously had tested positive with RT-PCR results did not show the presence of antibodies for the length of the study (at least 40 days after the initial onset of symptoms) (Zhao et al., 2020).

Ideally, any rapid diagnostic test for the outpatient setting must be accurate and reliable. Current research indicates that the diagnostic window for IgA and IgM is very limited. Some data indicate that host antibody testing can also yield inaccuracies. Also, for IgG testing, the significance of positive results is questionable at the current time. A positive result could indicate a previous infection, assuming the test did not cross-react with any other IgG the host produced in response to one of the four coronaviruses known to cause the common cold in humans, for example. It is not currently known,

however, if the presence of IgG antibodies indicates immunity (or degree thereof) of the host against SARS-CoV-2. The duration of any conferred immunity, or the level of IgG antibodies required to effectively acquire such immunity, are also unknown. Additional research is needed and encouraged.

Lisboa Bastos et al. (2020) performed a meta-analysis to investigate the diagnostic accuracy of serological testing for COVID-19. The authors aimed to identify studies where serological testing was compared to the "reference standard of viral culture or reverse transcriptase polymerase chain reaction". The authors identified a total of 40 studies for inclusion in the study. The authors found the pooled sensitivity of enzyme linked immunosorbent assays (ELISAs) measuring IgG or IgM to be 84.3% (with a 95% confidence interval [CI] of 75.6%-90.9%). For lateral flow immunoassays (LFIAs), the pooled sensitivity was found to be 66% (95% CI: 49.3%-79.3%), and for chemiluminescent immunoassays (CLIAs), the pooled sensitivity was found to be 97.8% (95% CI: 46.2%-100%). Pooled specificities ranged from 96.6%-99.7%. Sensitivity was also found to be higher at least three weeks from symptom onset (69.9% to 98.9%) compared to within the first week (13.4% to 50.3%) Of the samples used to calculate specificity, 83% were "from populations tested before the epidemic or not suspected of having COVID-19". The authors performed 49 bias risk assessments (one for methodology and one for patient selection) and identified 48 with a "high risk of patient selection bias" and 36 with "high or unclear risk of bias from performance or interpretation of the serological test". The authors also noted that only four of the forty studies including outpatients and only two studies evaluated point-of-care testing. The authors concluded that "currently, available evidence does not support the continued use of existing point-of-care serological tests" but acknowledged that "higher quality clinical studies assessing the diagnostic accuracy of serological tests for covid-19 are urgently needed" (Lisboa Bastos et al., 2020).

Kontou et al. (2020) performed a meta-analysis investigating the use of antibody tests in detecting SARS-CoV-2. The authors focused on IgG and IgM tests based on enzyme-linked immunosorbent assays (ELISA), chemiluminescence enzyme immunoassays (CLIA), fluorescence immunoassays (FIA), and lateral flow immunoassays (LFIA). A total of 38 studies encompassing 7848 individuals (3522 COVID-19 cases, 4326 healthy controls) were included. Of the 38 studies, 21 included data for both COVID-19 cases and controls. Fourteen studies using ELISA were included, and the authors found that IgG and IgM perform "similarly" individually, but in combination, resulted in a sensitivity of 0.935. Thirteen studies using CLIA resulted in an IgG sensitivity of 0.944, an IgM sensitivity of 0.810, and a combined IgG/IgM sensitivity of 0.910. The specificities ranged from 0.954 to 0.984. Thirteen studies used LFIA and found the IgG and IgM sensitivities to range from 0.53-0.66. Combining IgG and IgM resulted in sensitivities of 0.78-0.83. The authors also attempted to analyze FIA-based studies but were unable to due to the paucity of studies (three identified). The authors concluded that ELISA- and CLIA-based testing performed better sensitivity-wise and that LFIA studies are "more attractive for large seroprevalence studies but show lower sensitivity". (Kontou et al., 2020)

Ko et al. (2020) investigated the differences in neutralizing antibody production between asymptomatic and "mild" symptomatic COVID-19 patients, compared to pneumonic COVID-19 patients. A total of 70 patients (15 asymptomatic, 49 mild symptomatic, and 6 pneumonic) were included. A microneutralization assay was performed, along with a FIA and ELISA. Neutralizing antibody production was observed in all the pneumonic patients, 93.9% of the mildly symptomatic patients, and 80% of the asymptomatic patients. Further, the entire pneumonic group showed "high" titer (defined as ≥ 1:80), while 36.7% of the mild group and 20% of the asymptomatic group showed high titer. Both the FIA (for

IgG) and ELISA detected anti SARS-CoV-2 at a high sensitivity (98.8% and 97.6% respectively). The authors concluded that "Most asymptomatic and mild COVID-19 patients produced the neutralizing antibody, although the titers were lower than pneumonia patients" (Ko et al., 2020).

Wu et al. (2020) investigated the association between levels of neutralizing antibodies (NAbs) and clinical characteristics in recovered COVID-19 patients. A total of 175 patients with "mild" symptoms of COVID-19 were included. The authors found that NAbs were detected in patients starting in days 4-6 and reached peak levels in days 10-15. NAbs were also found not to cross-react with SARS-associated CoV,but correlated with "spike-binding antibodies targeting S1, receptor binding domain, and S2 regions. The authors also noted that NAbs titers were "significantly" higher in 56 "older" patients (1537 [IQR, 877-2427]) and 63 "middle-aged" patients (1291 [IRQ, 504-2126]) compared to 56 "younger patients" (459 [IQR, 225-998]). The authors concluded that "…NAb titers to SARS-CoV-2 appeared to vary substantially. Further research is needed to understand the clinical implications of differing NAb titers for protection against future infection" (Wu et al., 2020).

Kweon et al. (2020) collected 97 samples from patients with COVID-19 to analyze the serologic profiles and time kinetics of IgG and IgM against SARS-CoV-2 using the AFIAS COVID-19 Ab (BodiTechMed, 2020) and the EDI™ Novel Coronavirus COVID-19 ELISA Kit (EpitopeDiagnostics, 2020). The AFIAS assay uses recombinant nucleocapsid protein as an antigen to determine IgG and IgM antibodies against SARS-CoV-2 within 20 minutes from whole blood, serum, or plasma. The EDI™ ELISA Kit uses the microplate-based enzyme immunoassay technique to detect antibodies by measuring the optical densities (ODs) of each well of immunocomplexes. To determine the kinetics of antibodies, studies were performed at different past symptom onset (PSO) periods and to determine diagnostic accuracy of serologic assays, diagnostic sensitivity and specificities were calculated by PSO of ≤ 14 days and > 14 days. Kinetic studies showed that "with both assays, IgM and IgG rapidly increased after 7 days post symptom onset (PSO). IgM antibody levels reached a peak at 15–35 d PSO and gradually decreased. IgG levels gradually increased and remained at similar levels after 22–35 d" (Kweon et al., 2020). The diagnostic accuracy of both serologic assays also differed based on PSO. "The sensitivity of IgG samples from ≤14 d PSO was as low as 35.7%~57.1%, but it sharply increased for > 14 d PSO to 88.2%~94.1%. This means that almost all patients with COVID-19 showed seroconversion after 14 d PSO, and IgG seronegative subjects in this period are considered less likely to be infected with SARS-CoV-2. In addition, both assays showed 94.2~96.4% of IgG specificities and increased IgG titers in COVID-19 patients were maintained. Thus, IgG serologic assays can be useful for ruling out SARS-CoV-2 infection after 14 d PSO, detecting past infection, and epidemiologic surveys" (Kweon et al., 2020). For IgM, the sensitivities were "as low as 21.4% (same in both assays) in the samples collected ≤14 d PSO and 41.2%~52.9% in samples > 14 d PSO. These findings indicated that in patients infected with SARS-CoV-2, IgM seroconversion may not develop or might not be detected until the middle or late stages of infection. In other words, SARS-CoV-2 infection may be missed based on IgM seropositivity; thus, IgM tests must not be solely used in COVID-19 diagnosis and should be used only as a supportive tool in addition to molecular tests" (Kweon et al., 2020). In addition, IgM titers in COVID-19 patients showed a significant reduction after 35 d PSO; therefore, their utility in detecting past infection is limited. The author concludes that "testing for antibodies against SARS-CoV-2, especially IgG, has the potential for ruling out SARS-CoV-2 infection after 14 d PSO, detecting past infection, and epidemiologic surveys" (Kweon et al., 2020).

Caturegli et al. (2020) performed a case-control study to determine the clinical utility and validity of using SARS-CoV-2 antibodies, which were serum IgG and IgA antibodies formed against the SARS-CoV-2 spike protein detected by enzyme-linked immunosorbent assay (ELISA). When assays were formed 14 days or later after symptom onset, the researchers found that the sensitivity was 0.976 (95% CI, 0.928 to 0.995) and specificity was 0.988 (95% CI, 0.974 to 0.995), but the sensitivity decreased at earlier time points. Antibodies "predicted the odds of developing acute respiratory distress syndrome, which increased by 62% (CI, 48% to 81%; P < 0.001) for every 2-fold increase in IgG." This demonstrates the linkage of antibodies used to measure clinical severity and for those who tested negative by NAAT but remained potentially COVID-positive.

In a household cohort study, Churiwal assessed the utility of a rapid point of care test for COVID-19 antibodies by comparing the performance of BioMedomics COVID-19 IgM/IgG Rapid Antibody Test against an ELISA. The test was performed on 303 patients at study enrollment and 4 weeks later. According to the results, sensitivity was lower early in infection and those who never developed symptoms (74% sensitivity). Only two were detected among 499 tests early in infection due to false-positive IgM bands. When measured 4 weeks later after the onset of symptoms, it demonstrated robust sensitivity (90%) and complete specificity (100%). The authors conclude that "When used appropriately, rapid antibody tests offer a convenient way to detect symptomatic infections during convalescence" (Churiwal et al., 2021).

Antigen Testing

Another possible diagnostic testing methodology is antigen detection testing, which relies upon the direct detection of parts of the virus called "antigens"—in this instance, proteins located on the outside of SARS-CoV-2, such as the spike protein (S) or nucleocapsid protein, that can cause an immune response in an individual. What makes this method of testing distinct from antibody testing is that antigen testing directly measures the presence of the virus in a person whereas antibody testing is measuring the patient's response to an infection. These antigen detection tests can be deployed as rapid antigen tests that decrease the turnaround time for results but usually lack specificity (Loeffelholz & Tang, 2020).

On May 8, 2020, the FDA issued the first EUA for antigen testing for COVID-19 to the Quidel Corporation for their Sofia®2 SARS Antigen FIA lateral flow immunofluorescent sandwich assay for the qualitative detection of the nucleocapsid (N) protein antigen of SARS-CoV-2 for use in individuals suspected of COVID-19 by their healthcare provider (Quidel_Corporation, 2020). This test has been approved as a point-of-care (POC) test (FDA, 2022c). This test functions by detecting the N protein of either the SARS-CoV or SARS-CoV-2 virus from an upper respiratory sample (either a nasal swab or nasopharyngeal swab). First, the sample is placed in a reagent tube so that any virus, if present, is broken apart to allow for the N proteins to be exposed. The sample then travels from the sample well, down a test strip—where the term "lateral flow" is derived—where the proprietary reagents will recognize any N proteins and trap them in place on the strip. The test requires at least 15 minutes to develop prior to analysis. The strip can then be read by the Sofia®2 system that measures the fluorescent signal from the proprietary reagents. The Sofia®2 system allows the user to have two different modes for analysis—"Walk Away" and "Read Now". For the "Walk Away" mode, the user will insert the test cassette strip into the system, and the results will be displayed in 15 minutes because the test will be developed while

in the instrument. In "Read Now" mode, the user must have already allowed at least 15 minutes for the test to develop prior to inserting it into the instrument. Then, the Sofia®2 system will display the result within one minute (Quidel_Corporation, 2020). On August 20, 2020, Quidel reported that the Sofia test's labeling had been amended to include "either nasal or nasopharyngeal swabs" thereby allowing Quidel a second corresponding kit configuration (BioSpace, 2020).

On July 2, 2020, a second antigen test (BD Veritor System for Rapid Detection of SARS-CoV-2) from Becton, Dickinson, and Company was issued an EUA. This test is described as "a chromatographic digital immunoassay intended for the direct and qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasal swabs from individuals who are suspected of COVID-19 by their healthcare provider within the first five days of the onset of symptoms". The test is authorized for use in point-of-care (POC) settings. The test's mechanism of action is as follows: if there are any antigens in the sample (in this case, the nucleocapsid of the virus), they will bind to antibodies conjugated to detector particles in the test strip. The new "conjugates" migrate to the "reaction area" and are captured by another line of antibodies. The test reads positive when the conjugate is found at both "Control" and "Test" positions on the device. BD Veritor reported the following values for the test (in comparison to RT-PCR): 84% positive predictive agreement, 100% negative predictive agreement, 98% overall percent agreement, 100% positive predictive value, and 97.5% negative predictive value. No cross-reactivity was reported (BD_Veritor, 2020).

On August 18, 2020, a third antigen test (LumiraDx SARS-CoV-2 Ag Test from LumiraDx UK Ltd.) was issued an EUA. The test is described as "a single use fluorescence immunoassay device designed to detect the presence of the nucleocapsid protein antigen directly from SARS-CoV-2 in nasal swab specimens, without transport media". The mechanism of action is as follows: when a droplet of the specimen is added to the "Test Strip", pre-made reagents on the strip react with any antigen in the specimen. The amount of fluorescence created is proportional to the amount of antigen detected. LumiraDx reported a limit of detection of 32 $TCID_{50}$/mL [tissue-culture infectious dose], as well as a 97.6% positive percent agreement, 96.6% negative percent agreement, 93.1% positive predictive value, 98.8% negative predictive value, and 96.9% overall percent agreement (based on 257 total samples) (LumiraDx, 2020).

As of April 20, 2022, 50 antigen tests have Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA) (FDA, 2022d). These testing methods include (among others): Bulk Acoustic Wave (BAW) Biosensors, Chemiluminescence Immunoassays, Chromatographic Digital Immunoassays, Digital Lateral Flow, Magnetic Force-assisted Electrochemical Sandwich Immunoassay (MESIA), Microfluidic Immunofluorescence Assay, and Paramagnetic Microbead-based Immunoassay (FDA, 2022d).

Clinical Utility and Validity of Antigen Testing

To address the clinical performance, two primary studies were performed. Both studies only used frozen samples. The first study used 143 samples with 80% PPA or Positive Percent Agreement (47/59 of positive samples tested "positive"). They report 100% NPA or Negative Percent Agreement—all 84 negative samples tested "negative". The second study used a total of 48 samples. Again, 80% of the positive samples tested "positive"; however, only a total of five positive samples were included within

this second study. The remaining 43 samples were all negative samples. This study reports a sensitivity of 80.0%, but a 95% confidence interval range of 37.6% - 96.4%. A third supportive study was also performed. In this study, thirty swabs were taken. Twenty of these swabs were spiked with one lower concentration of the virus while the remaining ten swabs were spiked with a higher concentration of the virus. Then, all 30 swabs were tested and compared to 47 control ("unspiked") samples. In this study, none of the "unspiked" control samples tested "positive" while all 30 of the "spiked" samples, regardless of the concentration, tested positive. Quidel also tested the limit of detection (LoD) of the Sofia®2 SARS Antigen FIA test. LoD is typically measured by determining the $TCID_{50}$ (median tissue culture infective dose). The $TCID_{50}$ is the amount where 50% of the cells within a sample are infected.(Wulff et al., 2012) For the Sofia®2 SARS Antigen FIA test, the LoD for a direct swab sample has a $TCID_{50}$ of 113 mL whereas it is 850 mL if the initial sample is from a swab sample that has been diluted into 3 mL of reagent. Finally, Quidel also checked this antigen test for possible cross-reactivity with a number of microorganisms and other viruses. It shows no cross-reactivity with any of the microorganisms or viruses tests other than SARS-CoV. Of note, it does not cross-react with human coronavirus 229e, OC43, NL63, or MERS-CoV (heat-inactivated); however, they did not check for possible cross-reactivity with the other known human coronavirus (HKU1) due to a lack of availability at this time. This is noteworthy since this coronavirus is associated with the common cold. Limitations of the Sofia®2 SARS Antigen FIA test includes the following:

- This test must be performed using the Sofia®2 system, and the test must be performed accurately following the test procedure. Failure to do so can adversely affect the performance of the test and may invalidate the results.
- A positive test cannot distinguish between a SARS-CoV or a SARS-CoV-2 infection. SARS-CoV is the virus that caused the SARS outbreak of 2003. It should be noted that there is no current outbreak of SARS.
- This test also does not distinguish between "live" (viable) virus and non-viable virus. Consequently, the test results do not necessarily correlate with viral culture results performed on the same sample.
- This test is only for the qualitative use on a sample from either a nasal swab or a nasopharyngeal swab. It has not been approved for use, at this time, on any other sample, such as saliva.
- Negative test results can occur if the viral level is below the lower limit of the test. All negative results "should be treated as presumptive and confirmed with an FDA authorized molecular assay, if necessary, for clinical management, including infection control"(Quidel_Corporation, 2020)
- Positive test results do not rule out co-infections, and negative results do not "rule in" other non-SARS viral or bacterial infections.
- The clinical performance assays submitted for FDA approval were performed using frozen samples; the test may have a different performance when used with a fresh sample (such as in a point-of-care setting).
- "If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments is required (Quidel_Corporation, 2020)."
- As previously noted, the company did not check this test (as of publication date) for cross-reactivity with human coronavirus HKU1 due to a lack of availability of that strain. This is notable since this particular virus is associated with upper respiratory conditions such as the common

cold.

One multi-center study, currently a preprint at the time of publication, reports the development of another rapid antigen detection test (RADT) that screens for SARS-CoV-2 by targeting the nucleocapsid protein. This test, when using a nasopharyngeal swab sample, reports a 100% positive agreement with RT-PCR testing. They also report 73.6% positive agreement when using a urine sample (Diao et al., 2020). This study is yet to be published in a peer-reviewed journal, and the test is not FDA-approved as of May 18, 2020. Another study published recently in *ACS Nano* reports on the development of a RADT using field-effect transistor (FET)-based biosensing where a graphene sheet for the FET is coated with a specific antibody against the SARS-CoV-2 spike protein. This method can detect the protein in concentrations as low as 1 fg/mL in buffer and has an LOD of 242 copies/mL for a clinical sample (versus 16/mL for a culture medium) (Seo et al., 2020). To date, the WHO states that "Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19. Currently, there is insufficient evidence on performance and operational use to recommend specific commercial products" (WHO, 2021a).

Scohy et al. (2020) evaluated the Coris COVID-19 Ag [Antigen] Respi-Strip test in comparison to RT-PCR. The authors tested 148 nasopharyngeal swabs, with 106 testing positive by RT-PCR. The rapid antigen test detected 32 of these 106 positive results, for a sensitivity of 30.2%. All samples deemed positive by the antigen test were also deemed positive by RT-PCR. The authors noted that higher viral loads were associated with better detection by antigen tests but concluded that "the overall poor sensitivity of the COVID-19 Ag Respi-Strip does not allow using it alone as the frontline testing for COVID-19 diagnosis" (Scohy et al., 2020).

Mak et al. (2020) evaluated the BIOCREDIT COVID-19 Ag test in comparison to RT-PCR. The BIOCREDIT test's limit of detection (LOD) was compared to RT-PCR and viral culture, and a total of 368 samples from confirmed COVID-19 cases were included. A sample volume of 100 µL was used. The authors found the LOD of BIOCREDIT to be 1000-fold less sensitive than viral culture (BIOCREDIT LOD: 10^{-2}, viral culture: 10^{-5}). RT-PCR's LOD was measured to be 10^{-7}. Further, BIOCREDIT detected between 11.1% and 45.7% of RT-PCR positive patients from COVID-19 patients. The authors concluded that "This study demonstrated that the RAD test serves only as adjunct to RT-PCR test because of potential for false-negative results" (Mak et al., 2020).

Lambert-Niclot et al. (2020) analyzed the COVID-19 Ag Respi-Strip test and compared its accuracy to RT-PCR. A total of 138 nasopharyngeal samples were included, with 94 testing positive by RT-PCR. The Respi-Strip test identified 47 of 94 positive specimens for a sensitivity of 50%, although the specificity was 100% for both tests. The authors also noted that the control lines were "barely" visible for 17 tests (9 positive and 8 negative). The authors acknowledged that due to the low prevalence in France (the country in which this study was performed), prospective studies should be undertaken(Lambert-Niclot et al., 2020).

Hirotsu et al. (2020) evaluated a new antigen test (LUMIPULSE) which is based on chemiluminescence enzyme immunoassay. A total of 313 nasopharyngeal swabs were included (82 serial samples from 7 COVID patients, 231 individual samples from 4 COVID patients and 215 healthy controls). These samples were tested by both LUMIPULSE and RT-PCR. Compared to RT-PCR, LUMIPULSE demonstrated a 91.4% overall agreement rate (286/313), with a 55.2% sensitivity and 99.6% specificity. At > 100 viral copies,

LUMIPULSE agreed perfectly with RT-PCR, and at 10-100 viral copies, there was an 85% concordance rate (with concordance declining at lower viral loads). The authors concluded that "the LUMIPULSE antigen test can rapidly identify SARS-CoV-2-infected individuals with moderate to high viral loads and may be helpful for monitoring viral clearance in hospitalized patients" (Hirotsu et al., 2020).

Villaverde et al. (2021) conducted a multicenter study to compare the diagnostic accuracy of the Panbio coronavirus disease 2019 Antigen Rapid Test of nasopharyngeal samples in pediatric patients with COVID-19 symptoms ≤5 days. They demonstrated "limited accuracy in nasopharyngeal antigen testing: overall sensitivity was 45.4%, and 99.8% of specificity, positive-predictive value was 92.5%," with moderate concordance between the RT-PCR and antigen test. They noted that a high proportion of false-negative results from the antigen tests (54.5%) may have public health implications in unknown spreading of the virus. But because this test has a good positive likelihood ratio, and is cheap, rapid, and widely distributed, it may be used as a first screening test in a pandemic situation, though its value as a diagnostic tool is questionable due to the low sensitivity and negative likelihood ratio.

Peacock studied the clinical utility of the BinaxNOW antigen test by Abbott Diagnostics, a lateral flow immunochromatographic point-of-care test which provides results in 15 minutes from a nasal swab. BinaxNOW was performed on 735 samples and results were compared to PCR. 623 of 735 (84.8%) had symptoms and 460 of 623 patients (62.6%) had symptoms for less than 7 days. Positive tests occurred in 173 (23.5%) for the PCR and 141 (19.2%) with the BinaxNOW test. Those with symptoms for more than 2 weeks had a positive test rate half of those with earlier onset. " In patients with symptoms ≤7 days, the sensitivity, specificity, and negative and positive predictive values for the BinaxNOW test were 84.6%, 98.5%, 94.9%, and 95.2%, respectively" (Peacock et al., 2022). The authors conclude that BinaxNOW has good sensitivity and specificity and is recommended for patients with symptoms up to 2 weeks (Peacock et al., 2022).

Panel Testing

Multiple laboratories have developed panels to screen for possible microorganism infections from a single sample. For example, multiplex polymerase chain reaction (PCR) can simultaneously detect multiple pathogens rather than sequentially testing for each individual pathogen. Such testing can be advantageous when different pathogens may manifest with similar clinical presentation; however, this testing can be costly and can also result in false-negatives if preferential amplification of one target over another occurs (Palavecino, 2015). As of May 4, 2022, the BioFire® Respiratory Panel 2.1 (RP2.1), the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, ePlex Respiratory Pathogen Panel 2, cobas SARS-CoV-2 & Influenza A/B, Xpert Xpress SARS-CoV-2/Flu/RSV, Quest Diagnostics RC COVID-19 +Flu RT-PCR, Sofia 2 Flu + SARS Antigen FIA, and the Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay from the CDC received an EUA from the FDA for testing for COVID-19 (FDA, 2022c). The BioFire® Respiratory Panel 2.1, the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, and ePlex Respiratory Pathogen Panel 2 use multiplex nucleic acid testing from a nasopharyngeal swab to detect and differentiate microorganisms listed in **Table 1** (BioFire, 2020; GenMark_Diagnostics, 2020; Qiagen_GmbH, 2020), whereas the CDC Multiplex detects and differentiates influenzas A and B from SARS-CoV-2 (CDC, 2020c).

Table 1: Respiratory Pathogen Panel Testing Containing SARS-CoV-2

BioFire® Respiratory Panel 2.1	QIAstat-Dx® Respiratory SARS-CoV-2 Panel	ePlex Respiratory Pathogen Panel 2

• **Adenovirus**
• **HCoV 229E**
• **HCoV HKU1**
• **HCoVNL63**
• **HCoV OC43**
• **SARS-CoV-2**
• **Human Metapneumovirus**
• **Human Rhinovirus/Enterovirus**
• **Influenza A**
  ○ **Subtype H1**
  ○ **Subtype H3**
  ○ **Subtype H1-2009**
• **Influenza B**
• **Parainfluenza Virus 1**
• **Parainfluenza Virus 2**
• **Parainfluenza Virus 3**
• **Parainfluenza Virus 4**
• **Respiratory Syncytial Virus**
• ***Bordetella parapertussis***
• ***Bordetella pertussis***
• ***Chlamydia pneumoniae***
• ***Mycoplasma pneumoniae***
	• Adenovirus
	• HCoV 229E
	• HCoV HKU1
	• HCoVNL63
	• HCoV OC43
	• SARS-CoV-2
	• Human Metapneumovirus A+B
	• Influenza A
	  ○ Subtype H1
	  ○ Subtype H3
	  ○ Subtype 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*
	• *Chlamydia pneumoniae*
	• *Mycoplasma pneumoniae*
		• Adenovirus
		• HCoV 229E
		• HCoV HKU1
		• HCoVNL63
		• HCoV OC43
		• SARS-CoV-2
		• Human Metapneumovirus A+B
		• Influenza A
		  ○ Subtype H1
		  ○ Subtype H3
		  ○ Subtype H1-2009
		• Influenza B
		• Parainfluenza Virus 1
		• Parainfluenza Virus 2
		• Parainfluenza Virus 3
		• Parainfluenza Virus 4
		• Rhinovirus/Enterovirus
		• Respiratory Syncytial Virus A+B
		• *Chlamydia pneumoniae*
		• *Mycoplasma pneumoniae*

Clinical Utility and Validity of Panel Testing

The BioFire RP2.1 panel must be used with either the BioFire FilmArray 2.0 or BioFire FilmArray Torch Systems, and it does not provide a quantitative value for any particular organism within the sample. This panel "has not been established for specimens collected from individuals without signs or symptoms of respiratory infection (BioFire, 2020)." This panel has not been validated for the monitoring of treatment for any condition. If a test result shows four or more organisms detected, then the sample should be retested. A negative result does not necessarily exclude an infection. "Negative test results may occur from the presence of sequence variants (or mutation) in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, an infection caused by an organism not detected by the panel, or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen (BioFire, 2020)."

The BioFire RP2.1 panel cannot necessarily distinguish between existing viral strains and new variants.

One example is the inability to distinguish between Influenza A H3N2v and seasonal Influenza A H3N2. This panel also cannot reliably differentiate between human rhinovirus and enterovirus due to genetic similarity. If detected, the "result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required (BioFire, 2020)." The performance characteristics of several microorganisms detected by this panel, including HCoV 229E, were determined using retrospective clinical specimens due to the small number of positive specimens collected. The BioFire RP2.1 panel should not be used if *B. pertussis* is suspected because of its low sensitivity. "[A] *B. pertussis* molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to *B. pertussis* only should be used instead (BioFire, 2020)." This is because the RP2.1 panel targets a single-copy promoter target (*ptxP*) whereas more sensitive tests target the multi-copy IS*481* insertion sequence. The BioFire RP2.1 panel also shows cross-reactivity with *B. bronchiseptica* and *B. parapertussis* at higher concentrations.

The primers used in the BioFire RP2.1 panel to detect COVID-19 may cross-react with coronaviruses from other species due to high sequence homology. BioFire reports predicted cross-reactivity with up to three bat coronaviruses (accession: MN996532, MG772933, and MG772934) and one pangolin coronavirus (accession: MT084071). However, "[i]t is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (BioFire, 2020)."

The difference between the BioFire RP2 panel and the BioFire RP2.1 panel is the ability to detect SARS-CoV-2. Consequently, within the Instructions for Use (IFU) for the RP2.1 panel, BioFire reports on the data of the RP2 panel. The clinical performance of the RP2 panel was determined using both fresh and frozen samples. The clinical performance values for the four endemic HCoVs are listed in **Table 2** (BioFire, 2020). They note a cross-reactivity between HCoV-OC43 and HCoV-HKU1.

Table 2: Clinical Performance of BioFire RP2/RP2.1 Panel for Endemic HCoVs

Analyte	PPA	PPA 95% CI	NPA	NPA 95%CI
HCoV-229E	11/12 (91.7%)	64.6 – 98.5	1595/1600 (99.7%)	99.3 – 99.9
HCoV-HKU1	43/43 (100%)	91.8 – 100	1557/1569 (99.2%)	98.7 – 99.6
HCoV-NL63	40/40 (100%)	91.2 – 100	1562/1572 (99.4%)	98.8 – 99.7
HCoV-OC43	33/41 (80.5%)	66.0 – 89.8	1566/1571 (99.7%)	99.3 – 99.9

Notes: Abbreviations used—PPA (Positive Percent Agreement); NPA (Negative Percent Agreement).

Concerning the detection of SARS-CoV-2, the BioFire RP2.1 panel reports a limit of detection (LoD), using the USA-WA1/2020 isolate, of 500 copies/mL when using a heat-inactivated virus. They report a 100% detection rate (20/20). This equates to 6.9 X 10^{-2} $TCID_{50}$/mL. They also tested the LoD using an infectious virus isolate obtained from the World Reference Center for Emerging Viruses and Arboviruses

(WRCEVA), contributed by the CDC. With this infectious sample, the LoD was determined to be 160 copies/mL (or 1.1 X 10^{-2} $TCID_{50}$/mL). Again, they report a 100% detection rate (20/20) (BioFire, 2020).

Similar to the BioFire panel test, the QIAstat-Dx Respiratory SARS-CoV-2 panel test by Qiagen is for use on a proprietary system, the QIAstat Dx Analyzer System. It is also a qualitative test approved for testing in "patients suspected of COVID-19 by their healthcare provider". It is also "not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions" (Qiagen_GmbH, 2020). It is important to note that the test performance in either immunocompromised individuals or asymptomatic individuals has not been established as of publication date. A positive test result cannot rule out a co-infection; an erroneous negative test result can be due to erroneous sample handling as well as variations in the target sequences, organism levels below the limits of detection, and/or use of an interfering reagent (such as certain medications or therapies). Since the QIAstat-Dx test targets the *E* gene of SARS-CoV-2, which is homologous to sequences in multiple bat SARS viruses, it is possible to cross-react with these bat SARS viruses; however, the likelihood of infection of these viruses in humans is unlikely since none have been reported to date (Qiagen_GmbH, 2020).

Also, like the BioFire RP2/RP2.1 panel tests, the QIAstat-Dx test may not distinguish between existing viral strains and emerging viral strains, such as influenza A. However, unlike the BioFire RP2/RP2.1 panel tests, the QIAstat-Dx test does detect the IS*481* multi-copy insertion sequence present in multiple *Bordetella* species. This does increase the sensitivity of the test, but it can increase the possibility of false-positive results if the specimen is contaminated with a non-pertussis *Bordetella* species (Qiagen_GmbH, 2020).

In addressing the clinical performance of the QIAstat-Dx test for detecting SARS-CoV-2, Qiagen set up two positive trials (one at a higher concentration sample [n = 10] and one at a low positive contrived sample [n = 20), and they report a positive percent agreement (PPA) of 100% (30/30) (95% CI: 85.8 – 100%). Likewise, they did a negative control (n = 30) and report a negative percent agreement (NPA) of 100% (30/30) (95% CI: 85.8 – 100%). In reporting the limit of detection (LoD), they used 20 replicates with a detection rate of at least 95% (or 19/20) to generate a 'positive' signal. Using source material obtained from the clinical sample strain of the Hospital of Barcelona (Spain), Qiagen reports an LoD of 500 copies/mL.

The performance of the other targets within the panel were assessed in a multi-center study conducted at six geographically diverse study sites—Copenhagen, Denmark; Minneapolis, MN; Indianapolis, IN; Liverpool, NY; Columbus, OH; and Albuquerque, NM. The performance was determined using both frozen and fresh samples. The clinical performance values for the four endemic HCoVs are listed in **Table 3 (Qiagen_GmbH, 2020)**.

Table 3: Clinical Performance of QIAstat-Dx Panel for Endemic HCoVs

Analyte	PPA	PPA 95% CI	NPA	NPA 95%CI
HCoV-229E	8/9 (88.9%)	56.5 – 98.0	1975/1975 (100%)	99.8 – 100.0
HCoV-HKU1	51/52 (98.1%)	89.9 – 99.7	1925/1932 (99.6%)	99.3 – 99.8

HCoV-NL63	40/47 (85.1%)	72.3 – 92.6	1936/1938 (99.9%)	99.6 – 100.0
HCoV-OC43	26/29 (89.7%)	73.6 – 96.4	1951/1955 (99.8%)	99.5 – 99.9
Notes: Abbreviations used—PPA (Positive Percent Agreement); NPA (Negative Percent Agreement).				

As with the other two tests, the ePlex RP2 Panel "should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of active infection with the identified respiratory pathogen but do not rule out infection or co-infection with non-panel organisms. The agent detected by the ePlex RP2 Panel may not be the definite cause of disease. Negative results for SARS-CoV-2 and other organisms on the ePlex RP2 Panel may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Negative results do not preclude infection with SARSCoV-2 or other organisms on the ePlex RP2 Panel and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information (GenMark_Diagnostics, 2020)." A limitation of ePlex RP2 Panel is its unpredictability in differentiating human rhinovirus and enterovirus due to genetic similarity. If differentiation is required, an ePlex RP2 Panel positive human rhinovirus/enterovirus result should be followed-up using an alternative method, such as cell culture or sequence analysis. Cross-reactivity with SARS-CoV-1 is also observed at high titers.

To test the performance characteristics of ePlex RP2 Panel for SARS-CoV-2 detection, 170 nasopharyngeal previously frozen swab samples were collected (59 known SARS-CoV-2 positive and 111 presumed SARSCoV-2 negative samples). "Positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of TP and false negative (FN) results, while negative percent agreement (NPA) was calculated by dividing the number of true negative (TN) results by the sum of TN and false positive (FP) results" (GenMark_Diagnostics, 2020). The ePlex RP2 Panel detected SARS-CoV-2 in 59/59 positive specimens (100% positive percent agreement) and confirmed 111/111 negative specimens (100% negative percent agreement). To determine the limit of detection (LoD), the lowest concentration at which SARS-CoV-2 is detected at least 95% of the time, serial dilutions were prepared in a natural clinical matrix and at least 20 replicates per concentration were tested in the study. "The LoD concentration for detection of SARS-CoV-2 was determined to be 0.01 $TCID_{50}$/mL, which corresponds to 250 genomic copies per milliliter, as determined by digital droplet PCR (GenMark_Diagnostics, 2020)."

Regarding the "Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay" from the CDC, the FDA reported a limit of detection (LOD) of 1.01 x 10^{-2} (at ID_{50} [infective dose] / reaction). The panel was evaluated using 104 samples (33 positive for SARS-CoV-2, 30 positives for influenza A, and 30 positive for influenza B, 11 negative samples), and compared to an RT-PCR assay. There was a 100% concordance rate between the two tests. Additionally, cross-reactivity between the three analytes and 35 common respiratory pathogens (16 viruses, 18 bacterial species, 1 yeast) was evaluated, and no cross-reactivity was identified (FDA, 2020b).

The cobas SARS-CoV-2 & Influenza A/B panel is approved for emergency use authorization by the FDA; the panel uses qualitative detection of nucleic acids from SARS-CoV-2 in pooled samples. Six cultured viruses are tested for, two each of influenza A and influenza B strains as well as SARS-CoV-2. In an independent study, Poljak et al. (2020) performed a clinical evaluation of the cobas SARS-Cov-2 test (non-inclusive of influenza A/B panel). The cobas SARS-CoV-2 test was evaluated against an in-house and well-characterized comparator using 217 samples. cobas and the comparator showed overall agreement of 98.1%. Another comparative evaluation on 502 samples showed agreement of 99.6%. The authors concluded that cobas "is a reliable assay for qualitative detection of SARS-CoV-2 in nasopharyngeal swab samples collected in the Universal Transport Medium System (UTM-RT)(Poljak et al., 2020)."

There are other panels that are not yet FDA approved such as the AMPLIQUICK® Respiratory Triplex assay that detects and differentiates between SARS-CoV-2, influenza A/B and respiratory syncytial viruses in respiratory specimens. Results from AMPLIQUICK® were compared to the Allplex™ Respiratory Panel 1 and 2019-nCoV assays. 359 predetermined respiratory samples with diagnosed SARS-CoV-2, influenza A, influenza B and RSV were included in the study. The AMPLIQUICK® Respiratory Triplex "showed high concordance with the reference assays, with an overall agreement for SARS-CoV-2, influenza A, influenza B, and RSV at 97.6%, 98.8%, 98.3% and 100.0%, respectively." The authors conclude that the "AMPLIQUICK® Respiratory Triplex is a reliable assay for the qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and RSV in respiratory specimens, which may prove useful for streamlining diagnostics during the winter influenza-seasons" (Mboumba Bouassa et al., 2022).

Miscellaneous Testing

Other methodologies have been proposed to complement or even replace the standard tests described above. For example, a new "RT-LAMP" (reverse transcription loop-mediated isothermal amplification) application has started to see some use for the COVID-19 pandemic. This technique attempts to combine the speed of antigen testing and the accuracy of nucleic acid testing; RT-LAMP includes the traditional reverse transcriptase (RT), as well as a DNA polymerase with "strong strand displacement activity and tolerance for elevated temperatures and up to six DNA oligonucleotides of a certain architecture". These oligonucleotides act as primers for the RT, but additional oligonucleotides for the DNA polymerase are designed so that the DNA products loop back into their ends. This results in "self-priming templates" for the DNA polymerase, which allows the reaction [the nucleic acid amplification] to proceed as normal. Detection of the amplified DNA without specialized instrumentation is the key challenge; some tests use a pH indicator that changes the color of the solution the reaction is run in. Since the reaction does not require the use of a thermal cycler with real-time fluorescence measurement, the results can be delivered in a faster time frame than traditional RT-PCRs (Dao Thi et al., 2020).

Nagura-Ikeda et al. (2020) evaluated the "clinical performance of six molecular diagnostic tests and a rapid antigen test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)". Self-collected saliva was the medium used for analysis. A total of 103 patients with COVID-19 were included (15 asymptomatic, 88 symptomatic). The six molecular diagnostic tests included three RT-PCR tests, an RT-qPCR test, a "cobas SARS-CoV-2 high-throughput system" and an RT-LAMP assay. The molecular diagnostic tests detected viral RNA in 50.5%-81.6% of specimens and an antigen was detected in 11.7% of the specimens by the rapid antigen test. Viral RNA was also detected at a higher rate (65.6%-93.4%)

in specimens collected within 9 days of symptom onset compared to specimens collected after 10 days (22.2%-66.7%). Viral RNA was detected in asymptomatic patients at a rate of 40%-66.7%. The authors concluded "Self-collected saliva is an alternative specimen option for diagnosing COVID-19. LDT RT-qPCR…and RT-LAMP showed sufficient sensitivity in clinical use to be selectively used according to clinical settings and facilities. The rapid antigen test alone is not recommended for initial COVID-19 diagnosis because of its low sensitivity" (Nagura-Ikeda et al., 2020).

Dao Thi et al. (2020) performed a validation of a "two-color RT-LAMP assay protocol for detecting SARS-CoV-2 viral RNA using a primer set specific for the N gene". The authors wrote that a positive sample would be detected by a color change from red to yellow and tested their RT-LAMP assay on "surplus RNA samples isolated from 768 pharyngeal swab specimens collected from individuals being tested for COVID-19". The results were compared to a traditional RT-qPCR assay. The specificity of the RT-LAMP assay was found to be 99.7%. Further, the RT-qPCR positive samples with a cycle threshold (CT) number of under 30 scored positive (agreeance) in the RT-LAMP assay at a 97.5% agreeance rate. Agreeance rate declined both at the 30-35 threshold and at the 35-40 threshold. The authors also developed a "swab-to-RT" LAMP protocol, which was measured at 86% sensitivity (for CT < 30) and a 99.5% specificity. The authors concluded that "The RT-LAMP assay and LAMP-sequencing extend the range of available test methods and complement individual tests and pooled tests based on RT-qPCR with a faster, simpler, and potentially more cost-effective test method" (Dao Thi et al., 2020).

R. Wang et al. (2020) demonstrated a one-pot visual SARS-CoV-2 detection system named "opvCRISPR" by integrating reverse transcription loop-mediated isothermal amplification (RT-LAMP) and Cas12a cleavage in a single reaction system, which simplifies operations and avoids contamination. The opvCRISPR enables detection at every single molecular level in forty-five minutes. "The RT-LAMP reagents are incubated at the bottom of the tube, and CRISPR/Cas12a reaction reagents are added on the lid. SARS-CoV-2 RNA templates extracted from the respiratory swab are amplified by RT- LAMP, followed by mixing with the Cas12a reagents for cleavage. Once the Cas12a nuclease is activated by recognizing DNA target, it splits the quenched fluorescent single-stranded DNA (ssDNA) reporter (FAM- TTATT-BHQ1) indiscriminately, generating the fluorescence signal visible to the naked eye under blue light" (R. Wang et al., 2020). To investigate the diagnostic accuracy of opvCRISPR, 26 SARS-CoV-2 RT-PCR positive respiratory swab samples and 24 SARS-CoV-2 RT-PCR negative samples were tested. "All infected samples were determined to be SARS-CoV-2 positive while all uninfected samples tested to be negative by both opvCRISPR and RT- PCR. The opvCRISPR diagnostic results provide 100% agreement with the Centers for Disease Control and Prevention (CDC)-approved quantitative RT-PCR assay" (R. Wang et al., 2020). The author states that "the proposed method only requires minimal equipment, demonstrating great potential in enabling next-generation molecular diagnosis towards point-of- care diagnosis. However, the present method requires additional step to extract RNA. Further efforts need to be made to combine the RNA extraction module with the opvCRISPR to achieve from sampling to result nucleic acid detection" (R. Wang et al., 2020).

Another methodology with potential application for COVID-19 testing is next-generation sequencing (NGS). The NGS procedure typically includes the following steps: first the patient's DNA is prepared to serve as a template, then DNA fragments are isolated (on solid surfaces such as small beads) where sequence data is generated, then these results are compared against a reference genome. Any DNA

sample may be used if the quality and quantity of that sample are sufficient, but the methods of library generation and data analysis often vary from panel to panel. NGS is often used to produce swift and high-volume sequencing (Hulick, 2020). The FDA issued an EUA to Illumina, Inc. for the Illumina COVIDSeq Test on June 10, 2020 but has since updated its indications on October 29, 2020 to be for the "qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider" (FDA, 2021b). The FDA also issued an EUA to Helix OpCo LLC (dba Helix) for the Helix COVID-19 NGS Test on August 6, 2020. The test detects the gene for the SARS-CoV-2 spike protein, as well as one internal control (the human gene *RPP30*). The limit of detection was found to be 125 genetic copy equivalents / mL, and both the positive and negative percent agreements were measured to be 100% over 30 samples (Helix, 2020).

Furthermore, whole genome sequencing (WGS) has been demonstrated to have application for COVID-19 testing as well. WGS is conducted through four steps of DNA shearing, by using "molecular scissors" to cut DNA; then DNA bar-coding, for which "scientists add small pieces of DNA tags/bar codes to identify which piece of sheared DNA belongs to which [pathogen];" then the bar-coded DNA is put into the whole genome sequencer that identifies the bases; and finally, the data is analyzed to compare sequences and identify possible differences (CDC, 2016). In several countries, like the Netherlands, China, Vietnam, and the United States, particularly rapid WGS has been beneficial in informing outbreak response, general public health decision making, and infection risk in various facilities (Chau et al., 2021; Oude Munnink et al., 2020; Taylor et al., 2020; F. Wang et al., 2020). In the Netherlands, WGS with the first cases in February 2020 was able to confirm separate introductions of the virus into the country, and attribute increases in case prevalence to co-circulating virus variants following the spring holidays. WGS informed the sequence diversity that existed in Italy, which was where most COVID-positive individuals were returning from. The researchers concluded that "WGS in combination with epidemiological data strengthened the evidence base for public health decision-making in the Netherlands as it enabled a more precise understanding of the transmission patterns in various initial phases of the outbreaks. As such, we were able to understand the genetic diversity of the multiple introduction events in phase 1, the extent of local and regional clusters in phase 2 and the transmission patterns within the HCW [healthcare worker] groups in phase 3 (among which the absence or occurrence of very limited nosocomial transmission)" (Oude Munnink et al., 2020). In Vietnam, a similar application was made regarding a previously known strain responsible for a virus outbreak in the northern region. By whole genome sequencing, researchers were able to identify the first case of the B.1.1.7 variant from locally acquired infection. As the outbreak expanded, whole genome sequencing enabled enhanced surveillance in high risk groups, like those working in airports, who ended up being assigned another variant of A.23.1, as well as contact tracing and testing to detect more cases (Chau et al., 2021). In China, whole genome sequencing in this initial genomic study was able to provide insight towards the genotype-phenotype differences between COVID-19 positive patients. The researchers concluded, "Pedigree analysis suggested a potential monogenic effect of loss of function variants in GOLGA3 and DPP7 for critically ill and asymptomatic disease demonstration. Genome-wide association study suggests the most significant gene locus associated with severity were located in TMEM189–UBE2V1 that involved in the IL-1 signaling pathway…We identified that the HLA-A*11:01, B*51:01, and C*14:02 alleles significantly predispose the worst outcome of the patients" (F. Wang et al., 2020).

In the United States, a *Morbidity and Mortality Weekly Report (MMWR)* released in September 2020 utilized serial testing and virus whole genome sequencing at two skilled nursing facilities with COVID-19 outbreaks from April to June 2020 in Minnesota. From a total of 25 specimens from residents at the two different facilities, "strains from 17 residents and five HCP [health care personnel] were genetically similar, including one collected from a dietary worker with limited resident contact. Specimens from two HCP and one resident at facility A had distinctly different virus sequences from the first cluster and from each other. At facility B, 75 (66%) resident specimens and five (7%) HCP specimens were sequenced, all of which were genetically similar," which suggested "intrafacility transmission." However, the limited participation by HCPs in serial testing could have "have biased identification of infections and limited interpretation of genomic sequencing" and limited "the description of genetic diversity" (Taylor et al., 2020). Generally, whole-genome sequencing still seems to have some limitations, in that "it still presents practical difficulties such as high cost, shortage of available reagents in the global market, need of a specialized laboratorial infrastructure and well-trained staff" resulting in "SARS-CoV-2 surveillance blackouts across several countries" (Bezerra et al., 2021). As of May 4, 2022, there are no FDA approved tests specifically for WGS.

Other types of specimens or media have been proposed as viable for COVID-19 testing, such as saliva. Saliva's primary advantages include its flexibility, its safety, and overall ease of use in testing. Santosh et al. also noted that To et al. found that saliva has a "high consistency rate of greater than 90% with nasopharyngeal specimens in the detection of respiratory viruses, including coronaviruses" (Sri Santosh et al., 2020; To et al., 2019). On August 15, 2020, the FDA issued an EUA to Yale School of Public Health for "SalivaDirect" which uses saliva samples for COVID-19 testing. Although this test still uses RT-PCR, the test still detects the nucleic acids in saliva, but does not require otherwise specialized or proprietary equipment for extraction of those nucleic acids. In the "Performance Evaluation" section of the official EUA, the FDA noted a positive agreement level between SalivaDirect and the ThermoFisher Scientific TaqPath COVID-19 combo kit to be 94.1% (32/34) and a negative agreement level to be 90.9% (30/33). (FDA, 2020a)

A third innovation in COVID-19 testing was published by the FDA on July 18, 2020. On this date, the FDA stated that they reissued an EUA to Quest Diagnostics to authorize Quest SARS-CoV-2 rRT-PCR test for use with "pooled" samples. This testing practice refers to testing multiple samples simultaneously, thereby allowing more efficient testing. The Quest SARS-CoV-2 rRT-PCR test was authorized to test up to 4 samples at once. The FDA notes that this strategy is most efficient in areas with low prevalence of COVID (i.e., most tests are expected to be negative). In the EUA, the FDA writes that if the "positivity rate" for any given individual to be tested is over 25%, the pooling strategy should not be used due to inefficiency (FDA, 2020c). Yelin et al. found that a single positive sample could be identified in pools of up to 32 samples (with a false negative rate of 10%) and noted that detection of a single positive sample in a pool of 64 samples may be possible with additional amplification cycles. (Yelin et al., 2020). Additional EUAs have been issued specifically for tests using pooled samples, such as the UCSD RC SARS-CoV-2 Assay (University of California San Diego Health, RT-PCR, 5 samples), the Poplar SARS-CoV-2 TMA Pooling assay (Poplar Healthcare, TMA [transcription-mediated amplification], 7 samples), and the "COVID-19 RT-PCR Test" (LabCorp, RT-PCR, 5 samples) (LabCorp, 2020a; Poplar, 2020; UCSD, 2020).

Hogan et al. (2020) performed an analysis of pooled sample analysis in a community setting. The authors analyzed samples in pools of 9 or 10, and the RT-PCR assay targeted the envelope (E) gene. When a

positive pool was identified, each sample was tested individually for both the E gene and the RNA-dependent RNA polymerase (RdRp) gene for confirmation. The authors investigated 292 pools encompassing 2740 nasopharyngeal samples and 148 bronchoalveolar lavage samples. Two positive samples were identified (0.07%), which both showed detection of both genes. The authors identified one pool with a "positive E signal" that was not reproducible with testing individual samples of that pool. The authors did acknowledge that this methodology may miss individuals in which a COVID-19 risk has not been identified, but concluded that "strategies such as pooled screening may facilitate detection of early community transmission of SARS-CoV-2 and enable timely implementation of appropriate infection control measures to reduce spread (Hogan et al., 2020).

Another innovative technique in COVID-19 testing was announced on April 14, 2022 in an FDA press announcement. The InspectIR COVID-19 Breathalyzer is the first FDA Emergency Use Authorization-approved diagnostic test to use breath samples; the test detects chemical compounds in breath and provides results in less than three minutes. According to the FDA, a validation study of 2,409 individuals (both symptomatic and asymptomatic) showed 91.2% sensitivity and 99.3% specificity for detecting COVID-19. In addition, the study evidenced a negative predictive value of 99.6% in a population composed of only 4.2% of people who were positive for the virus. A follow-up study with the Omicron variant showed similar sensitivity values (FDA, 2022b).

IV. Guidelines and Recommendations

World Health Organization

The World Health Organization (WHO) published an interim guideline for the diagnostic testing of "2019 novel coronavirus [termed 2019-nCoV]" on September 11, 2020 (WHO, 2020a). First, they state that routine confirmation of COVID-19 cases is based on nucleic acid testing. Regarding serum testing, they remark that "if negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2-4 weeks later can be used to look for seroconversion or a rise in antibody titres." Finally, they recommend against viral culture or isolation as a routine diagnostic procedure and WHO does not recommend the use of saliva as the sole specimen type for routine clinical diagnostics (WHO, 2020a).

The WHO released a scientific brief with recommendations for the use of SARS-CoV-2 Ag-RDTs, and updated their interim guidance on October 6, 2021. Within the guidelines, "SARS-CoV-2 Ag-RDTs (antigen detecting rapid diagnostic tests) that meet the minimum performance requirements of ≥ 80% sensitivity and ≥ 97% specificity compared to a NAAT reference assay can be used to diagnose SARS-CoV-2 in suspected COVID-19 cases" (WHO, 2021a). Ag-RDTs should be conducted within 5-7 days after the onset of symptoms, as "patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher." WHO recommends that Ag-RDTs be used in settings when they are most reliable – in areas "when there is ongoing community transmission (≥ 5% test positivity rate). When there is no transmission or low transmission, the positive predictive value of Ag-RDTs will be low (many false positives), and in this setting NAAT is preferable as the first-line testing method or for confirmation of positive Ag-RDTs."

WHO recommends that the following populations be tested with Ag-RDTS:

does not recommend using SARS-CoV-2 Ag-RDTs when:

- "Symptomatic individuals (suspected COVID-19 cases) in the first 5-7 days since onset of symptoms"
- For asymptomatic individuals, only "limited to contacts of confirmed or probable cases and to at-risk health workers until more evidence is available on the benefits and cost effectiveness of testing low-risk groups with no known exposure to SARS-CoV-2, particularly in settings where testing capacity is limited."
- "Suspected COVID-19 cases in outbreak investigations"

The WHO also indicates the following as priority uses for the Ag-RDTs:

- "Community testing of symptomatic individuals meeting the case definition of suspected COVID-19."
- "To detect and respond to suspected outbreaks of COVID-19 including in remote settings, institutions and semi-closed communities (e.g., schools, care-homes, cruise ships, prisons, workplaces and dormitories), especially where NAAT is not immediately available."
- "To screen asymptomatic individuals at high risk of COVID-19, including health workers, contacts of cases and other at-risk individuals."

Overall, "Ag-RDT testing is recommended in settings likely to have the most impact on early detection of cases for care and contact tracing and where test results are most likely to be correct" (WHO, 2021a).

WHO released a second scientific brief with recommendations concerning immunity passports (WHO, 2020b) on April 24, 2020. Within the guidelines, WHO states that as of the publication date, "no study has evaluated whether the presence of antibodies to SARS-CoV-2 confers immunity to subsequent infection by this virus in humans." They go on to note, "Laboratory tests that detect antibodies to SARS-CoV-2 in people, including rapid immunodiagnostic tests, need further validation to determine their accuracy and reliability. Inaccurate immunodiagnostic tests may falsely categorize people in two ways. The first is that they may falsely label people who have been infected as negative, and the second is that people who have not been infected are falsely labelled as positive. Both errors have serious consequences and will affect control efforts. These tests also need to accurately distinguish between past infections from SARS-CoV-2 and those caused by the known set of six human coronaviruses. Four of these viruses cause the common cold and circulate widely. The remaining two are the viruses that cause Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome. People infected by any one of these viruses may produce antibodies that cross-react with antibodies produced in response to infection with SARS-CoV-2 (WHO, 2020b)".

In 2021, WHO released an update to the scientific brief concerning immunity passports within a document titled 'COVID-19 natural immunity.' Within this brief, WHO discusses the various testing methods available. WHO notes that "there are many available serologic assays that measure the antibody response to SARS-CoV-2 infection, but at the present time, the correlates of protection are not well understood". The most measured immune response is the presence of antibodies in serum. Serologic assays to detect the antibody response are usually based on enzyme immunoassays, which

detect the presence of virus-specific antibodies in the blood or by live or pseudo-virus neutralization assays, which detect functional NAb. While serologic testing has limited use in clinical management because it does not capture active infection, it can be very useful in determining the extent of infection or estimating attack rates in given populations. Interpreting the results of serologic testing, however, is complex: there are several antibody types and subtypes and multiple antigenic determinants/epitopes that can be used to target these antibodies, and the results may differ substantially depending on the combinations chosen. The results will also depend on the manufacturing specifics of the assay used" (WHO, 2021c). Other frequently used assays are enzyme-linked immunosorbent tests, chemiluminescent tests, and lateral flow rapid diagnostic tests. To conclude, "available tests and current knowledge do not tell us about the duration of immunity and protection against reinfection, but recent evidence suggests that natural infection may provide similar protection against symptomatic disease as vaccination, at least for the available follow up period."

WHO released a scientific brief on May 15, 2020, concerning multisystem inflammatory syndrome in children and adolescents with COVID-19. Within the guidelines, they recommend standardized data describing clinical presentations.

- WHO gives a preliminary case definition for individuals ages 0 – 19 years with fever three or more days AND at least TWO of the following:
  - "Rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs (oral, hands or feet).
  - Hypotension or shock.
  - Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including ECHO findings or elevated Troponin/NT-proBNP),
  - Evidence of coagulopathy (by PT, PTT, elevated d-Dimers).
  - Acute gastrointestinal problems (diarrhea, vomiting, or abdominal pain).
- AND
  - Elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin.
- AND
  - No other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes.
- AND
  - Evidence of COVID-19 (RT-PCR, antigen test or serology positive), or likely contact with patients with COVID-19 (WHO, 2020c)."

Centers for Disease Control and Prevention

In the update of the CDC guidelines *Overview of Testing for SARS-CoV-2,* dated February 11, 2022, the CDC states that "viral tests, including nucleic acid amplification tests (NAATs, such as Reverse Transcription-Polymerase Chain Reaction), antigen tests and other tests (such as breath tests) are used as diagnostic tests to detect current infection with SARS-CoV-2 and to inform an individual's medical care." The CDC also stated, "Viral testing is recommended for individuals who are close contacts of persons with COVID-19" (CDC, 2022n). Regarding use of antibody testing, the CDC remarks: "Antibody testing is not currently recommended to assess a person's protection against infection or severe COVID-19 following COVID-19 vaccination or prior infection, or to assess the need for vaccination in an

unvaccinated person. To evaluate for evidence of previous infection in a vaccinated individual, an antibody test specifically evaluating IgM/IgG to the nucleocapsid protein should be used (e.g., for public health surveillance or the diagnosis of Multisystem Inflammatory Syndrome in Children (MIS-C) Multisystem Inflammatory Syndrome in Adults (MIS-A)" (CDC, 2022n).

The interim guideline for COVID-19 testing in non-healthcare workplaces also describes individuals for which SARS-CoV-2 testing with viral tests (i.e., nucleic acid or antigen tests) is "appropriate", which include "all unvaccinated close contacts (people who have been within 6 feet for a combined total of 15 minutes or more during a 24-hour period)." For these close contacts, "because of the potential for asymptomatic (not having symptoms) or pre-symptomatic (not yet showing symptoms) transmission of SARS-CoV-2, it is important that unvaccinated individuals exposed to people with known or suspected COVID-19 be quickly identified and quarantined." Generally, for screening in the workplace, "given the incubation period for COVID-19 (up to 14 days), CDC recommends conducting screening testing of unvaccinated, asymptomatic workers without known or suspected exposures at least weekly." Diagnostic testing should be done for the following situations and populations (CDC, 2021b):

- "Persons with signs or symptoms consistent with COVID-19"
- "Asymptomatic persons with recent known or suspected exposure to SARS-CoV-2"
- "Determining resolution of infection (e.g., ending isolation)"

Screening testing considerations should also be implemented for "testing asymptomatic persons without known or suspected exposure to SARS-CoV-2 for early identification, isolation, and disease prevention." This guideline is currently inactive on the CDC website (CDC, 2021b).

Regarding testing for asymptomatic patients with "known or suspected exposure to SARS-CoV-2", the CDC recommends testing for all close contacts of persons with SARS-CoV-2 infection. "Viral testing is recommended for individuals who have been exposed persons with COVID-19. People who have had an exposure with someone known or suspected of having COVID-19 should be tested at least 5 days after the exposure. If symptoms develop before 5 days, they should get tested immediately" (CDC, 2022n). The CDC also notes the settings to prioritize for screening testing, including:

- "High-risk congregate settings, such as assisted living facilities, correctional facilities, and homeless shelters, that have demonstrated high potential for rapid and widespread virus transmission to people at high risk for severe illness."
- "Settings that involve close quarters and that are isolated from healthcare resources (e.g., fishing vessels, wildland firefighter camps, or offshore oil platforms)" (CDC, 2022n).

The CDC states it is "working with state, local, territorial, academic, and commercial partners" for surveillance testing and COVID-19 research in the US (CDC, 2022n). In general, the CDC remarks that viral testing is "diagnostic" when conducted among symptomatic patients, asymptomatic patients with known or suspected exposure to SARS-CoV-2, and to determine infection resolution. Viral testing is considered "screening" when performed on asymptomatic patients without known exposure to SARS-CoV-2 (CDC, 2021b).

Finally, the CDC still maintains, "Consequently, evidence supports a time-based and symptom-based strategy to determine when to discontinue isolation or other precautions rather than a test-based

strategy. For persons who are severely immunocompromised, a test-based strategy could be considered in consultation with infectious disease experts. For all others, a test-based strategy is no longer recommended" (CDC, 2021b).

The CDC also published *Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings* on August 1, 2020, and updated the guidelines on January 24, 2022. The CDC states that "Both SARS-CoV-2 IgM and IgG antibodies may be detected around the same time after infection. However, while IgM is most useful for determining recent infection, it usually becomes undetectable weeks to months following infection; in contrast, IgG may remain detectable for longer periods. IgA is important for mucosal immunity and can be detected in mucous secretions like saliva in addition to blood; although, its significance in this disease is still to be determined." The CDC also acknowledges the potential application of neutralizing antibody detection (as opposed to binding antibody detection), and remarks that the FDA has now authorized one competitive neutralization test (cVNT), which is a "binding antibody tests designed to qualitatively detect potentially neutralizing antibodies, often those that prevent interaction of RBD with the ACE-2 receptor" (CDC, 2022i).

Regarding testing for past infections, the CDC recommends that antibody tests should not be used to diagnose a current COVID-19 infection (CDC, 2022a).

Within the CDC's *Interim Guidelines for Collecting and Handling Clinical Specimens for COVID-19 Testing*, they recommend collecting and testing upper respiratory samples for initial diagnostic testing for current SARS-CoV-2 infections. Within their recommendation, they list the following (without stating a preference) as acceptable specimens:

- "Nasopharyngeal (NP) specimen collection/oropharyngeal (OP) (throat) specimen collection (performed by a trained healthcare provider, only)
- Nasal mid-turbinate (MT) swab (performed by a healthcare provider or the patient after reviewing and following collection instructions)
- Anterior nasal specimen (performed by a healthcare provider or the patient after reviewing and following collection instructions)
- Nasopharyngeal wash/aspirate or nasal wash/aspirate (NW) (performed by a trained healthcare provider)
- Saliva (collected by patient with or without supervision)
- Breath (performed by a qualified, trained operator under the supervision of a healthcare provider licensed or authorized by state law to prescribe tests)
- Bronchoalveolar lavage, tracheal aspirate, pleural fluid, lung biopsy (generally performed by a physician in the hospital setting)
- Sputum (collected under the guidance of a trained healthcare professional)" (CDC, 2022g).

The CDC issued employer-based guidelines titled *Guidance for Businesses and Employers Responding to Coronavirus Disease 2019 (COVID-19): Plan, Prepare, and Respond to Coronavirus Disease 2019*. (CDC, 2021a). This guideline is now considered archived and made available for historical purposes, and the CDC now refers to the OSHA guideline titled *Protecting Workers: Guidance on Mitigating and Preventing the Spread of COVID-19 in the Workplace* for the latest information.

The CDC, in a joint interim set of guidelines with OSHA, issued Meat and Poultry Processing Workers and

Employers Interim Guidance from CDC and the Occupational Safety and Health Administration (OSHA) (CDC & OSHA, 2021). This guideline is now considered archived and made available for historical purposes, and the CDC now refers to the OSHA guideline titled *Protecting Workers: Guidance on Mitigating and Preventing the Spread of COVID-19 in the Workplace*.

In their August 31, 2022 update, the CDC recommends a test-based strategy "may be used to remove a mask sooner." The CDC recommends a symptom-based strategy primarily for healthcare professionals. The recommendations are listed below:

- "For people who are mildly ill with SARS-COV-2 infection and not moderately or severely immunocompromised,
  - Isolation can be discontinued at least 5 days after symptom onset (day 0 is the day symptoms appeared, and day 1 is the next full day thereafter) if fever has resolved for at least 24 hours (without taking fever-reducing medications) **and** other symptoms are improving.
  - If symptoms recur or worsen, the isolation period should restart at day 0.
  - People who cannot wear a mask, including children < 2 years of age and people of any age with certain disabilities, should isolate for 10 days.
  - In certain high-risk congregate settings that have high risk of secondary transmission, CDC recommends a 10-day isolation period for residents."
- "For people who test positive, are asymptomatic (never develop symptoms) and not moderately or severely immune compromised,
  - Isolation can be discontinued at least 5 days **after the first positive viral test** (day 0 is the date the specimen was collected for the positive test, and day 1 is the next full day thereafter)."
- "For people who are moderately ill and not moderately or severely immunocompromised, isolation and precautions can be discontinued 10 days after symptom onset (day 0 is the day symptoms appeared, and day 1 is the next full day thereafter)."
- "For people who are severely ill and not moderately or severely immunocompromised, isolation and precautions can be discontinued 10 days after symptom onset (day 0 is the day symptoms appeared, and day 1 is the next full day thereafter).
  - Some people with severe (e.g., requiring hospitalization, intensive care, or ventilation support) may remain infectious beyond 10 days. This may warrant extending the duration of isolation and precautions for up to 20 days after symptom onset (with day 0 being the day symptoms appeared) **and** after resolution of fever for at least 24 hours (without taking fever-reducing medications) **and** improvement of other symptoms.
  - Serial testing prior to ending isolation can be considered in consultation with infectious disease experts."
- "For people who are moderately or severely immunocompromised (regardless of COVID-19 symptoms or severity), … CDC recommends an isolation period of at least 20 days, and ending isolation in conjunction with serial testing and consultation with an infectious disease specialist to determine the appropriate duration of isolation and precautions.
  - The criteria for serial testing to end isolation are:
    - Results are negative from at least two consecutive respiratory specimens collected In certain high-risk congregate settings that have high risk of secondary transmission, CDC recommends a 10-day isolation period for residents.
    - Also, if a moderately or severely immunocompromised patient with COVID-19 was

symptomatic, there should be resolution of fever for at least 24 hours (without the taking fever-reducing medication) and improvement of other symptoms" (CDC, 2022e).

In terms of ending isolation for nonhealthcare personnel, the recommendations are below, utilizing a symptom-based strategy as well:

- "If you had no symptoms, you may end isolation after day 5."
- "If you had symptoms and:
  - Your symptoms are improving – you may end isolation after day 5 if you are fever-free for 24 hours (without the use of fever-reducing medication).
  - Your symptoms are not improving – continue to isolate until: you are fever-free for 24 hours (without the use of fever-reducing medication) [and] your symptoms are improving.
- "If you had symptoms and had:
  - Moderate illness (you experienced shortness of breath or had difficulty breathing):
    - You need to isolate through day 10.
  - Severe illness (you were hospitalized) or have a weakened immune system:
    - You need to isolate through day 10.
    - Consult your doctor before ending isolation" (CDC, 2022j).

The CDC also published a case series of "Multisystem Inflammatory Syndrome in Adults Associated with SARS-CoV-2 Infection" [MIS-A]. The CDC observes that a "hyperinflammatory syndrome resembling MIS-C" may also manifest in adult patients and remarks that "Clinicians and health departments should consider MIS-A in adults with signs and symptoms compatible with the current working MIS-A case definition. Antibody testing for SARS-CoV-2 might be needed to confirm previous COVID-19 infection in patients who do not have positive SARS-CoV-2 PCR or antigen test results." The working case definition of MIS-A was defined by CDC as follows:

- "a severe illness requiring hospitalization in a person aged ≥ 21 years;
- a positive test result for current or previous SARS-CoV-2 infection (nucleic acid, antigen, or antibody) during admission or in the previous 12 weeks;
- severe dysfunction of one or more extrapulmonary organ systems (e.g., hypotension or shock, cardiac dysfunction, arterial or venous thrombosis or thromboembolism, or acute liver injury);
- laboratory evidence of severe inflammation (e.g., elevated CRP, ferritin, D-dimer, or interleukin-6); and
- absence of severe respiratory illness (to exclude patients in which inflammation and organ dysfunction might be attributable simply to tissue hypoxia)."

Patients with mild respiratory symptoms who met these criteria were included. Patients were excluded if alternative diagnoses such as bacterial sepsis were identified.

The CDC does note three limitations of the case series report, which are as follows:

- "First, cases described here were voluntarily reported or published and therefore are not representative of the true clinical spectrum or racial/ethnic distribution of this emerging syndrome. Additional cases might not have been reported or published; others might have remained unrecognized because of absence of COVID-like symptoms, lack of antibody testing, or negative test results.

- Second, the working case definition excludes patients with severe respiratory dysfunction to distinguish MIS-A from severe COVID-19; however, the two conditions might overlap in some cases.
- Finally, the working case definition for this syndrome is potentially nonspecific, and some patients with other disease processes might have been misclassified as having MIS-A" (Morris et al., 2020).

On July 9, 2021, the CDC updated their research on "Post-COVID Conditions: Information for Healthcare Providers" (CDC, 2022m). According to the CDC, "Post-COVID conditions" can be referred to as "long COVID, post-acute COVID-19, long-term effects of COVID, post-acute COVID syndrome, chronic COVID, long-haul COVID, late sequelae, and the research term "post-acute sequelae of SARS-COV-2 infection (PASC)." Generally, "it can be considered a lack of return to a usual state of health following acute COVID-19 illness" and "might also include development of new or recurrent symptoms unmasking of a pre-existing condition that occurs after the symptoms of acute illness have resolved." However, the CDC considers post-COVID conditions "to be present if recovery does not occur after the 4-week acute phase even though many patients continue to recover between 4 and 12 weeks." The CDC also warns of the possibility that some patients with post-COVID conditions won't have positive tests for COVID-19 because of "a lack of testing or inaccurate testing during the acute period, or because of waning antibody levels or false-negative antibody testing during follow up." Alternatively, the term "long COVID" is defined as "symptoms lasting 3 or more months that were not present prior to having COVID-19." The CDC cites the most common symptoms to include: "Dyspnea or increased respiratory effort, fatigue, post-exertional malaise and/or poor endurance, cognitive impairment or "brain fog", cough, chest pain, headache, palpitations and tachycardia, arthralgia, myalgia, paresthesia, abdominal pain, diarrhea, insomnia and other sleep difficulties, fever, lightheadedness, impaired daily function and mobility, pain, rash (e.g., urticaria), mood changes, anosmia or dysgeusia, menstrual cycle irregularities, [and] erectile dysfunction." "Post-exertional malaise (PEM)" is defined as "the worsening of symptoms following even minor physical or mental exertion, with symptoms typically worsening 12 to 48 hours after activity and lasting for days or even weeks" (CDC, 2022m).

Centers for Medicare & Medicaid Services

The Centers for Medicare & Medicaid Services (CMS) released recommendations regarding the reopening of nursing homes for state and local officials. Concerning testing, CMS recommends that each facility should have a plan that at a minimum consider the following:

- The capacity to test all nursing home residents and staff (including "individuals providing services under arrangement and volunteers") once with a single baseline COVID-19 test.
- Similarly, the capacity to "test any resident or staff who has signs or symptoms of COVID-19"
- "The capacity for all staff and residents to be tested upon identification of a single new case of COVID-19 infection in any staff or residents. Capacity for continuance of re-testing until testing identifies no new cases of COVID-19 infection among staff or residents for a period of at least 14 days since the most recent positive result."
- "The capacity for routine staff testing based on the facility's county-positivity rate."
- The capacity for all staff members, including all volunteers and vendors who are on site on a weekly basis, to receive a single baseline COVID-19 test and to have weekly re-testing of all staff. CMS notes that state and local governments may adjust the frequency of testing based on the

circulation of the virus in the community.
- Written screening protocols should be available for all staff, all residents, and all persons entering the facility, including vendors, volunteers, and visitors.
- "The ability to perform diagnostic testing or an arrangement with laboratories to process diagnostic tests for the SARS-CoV-2 virus, with results obtained rapidly (e.g., within 48 hours)."
- The tests used to screen for COVID-19 should have greater than 95% sensitivity and greater than 90% specificity with the results obtained rapidly (within 48 hours).
- Antibody testing should not be used for diagnosis of an active COVID-19 infection.
- Any staff or resident who either refuses or is unable to comply is to be considered positive (Wright & CMS, 2021).

In a "Frequently Asked Questions" publication from October 21, 2021, the CMS writes that the potential risks of using pooled samples include "a risk of obtaining false negative or false positive results when utilizing a pooled sampling testing model" and that "all positive and inconclusive SARS-CoV-2 results from pooled sampling must be confirmed by having each participant whose sample was contained within the cohort to be tested by a CLIA-certified facility." The CMS notes that they do not have oversight authority over facilities that are not CLIA-certified (CMS, 2021).

National Institutes of Health

The NIH released COVID-19 treatment guidelines, and within the September 26, 2022 update, they addressed clinical spectrum of SARS-CoV-2 infection, which includes those with asymptomatic or presymptomatic infection, mild illness, moderate illness, severe illness, and critical illness. For asymptomatic and presymptomatic individuals, the NIH states that "It is unclear what percentage of individuals who present with asymptomatic infection progress to clinical disease. Some asymptomatic individuals have been reported to have objective radiographic findings consistent with COVID-19 pneumonia." Additionally, the guideline discusses infectious complications in patients with COVID-19, which can be categorized as "coinfections at presentation," such as "concomitant viral infections, including influenza and respiratory viruses" and community-acquired bacterial pneumonia, and "reactivation of latent infections," such as chronic hepatitis B virus and latent tuberculosis reactivation, "nosocomial infections," such as hospital-acquired or ventilator-associated pneumonia and *Clostridioides difficile*-associated diarrhea, and "opportunistic fungal infections," like aspergillosis and mucormycosis among hospitalized COVID-19 patients (NIH, 2022a).

The NIH also released COVID-19 testing guidelines. The following recommendations were made from the COVID-19 Treatment Guidelines Panel:

- "To diagnose acute infection of SARS-CoV-2, the panel recommends "using either a nucleic acid amplification test (NAAT) or an antigen test with a sample collected from the upper respiratory tract (e.g., nasopharyngeal, nasal mid-turbinate, or anterior nasal) to diagnose acute infection of SARS-CoV-2 (AIII).
- A NAAT should not be repeated in an asymptomatic person (with the exception of health care workers) within 90 days of a previous SARS-CoV-2 infection, even if the person has had a significant exposure to SARS-CoV-2 (AIII).
- SARS-CoV-2 reinfection has been reported in people after an initial diagnosis of the infection;

therefore, clinicians should consider using a NAAT for those who have recovered from a previous infection and who present with symptoms that are compatible with SARS-CoV-2 infection if there is no alternative diagnosis (BIII).

- The Panel **recommends against** diagnosing acute SARS-CoV-2 infection solely on the basis of serologic (i.e., antibody) test results (AIII).
- There is insufficient evidence for the Panel to recommend either for or against the use of SARS-CoV-2 serologic testing to assess for immunity or to guide clinical decisions about using COVID-19 vaccines or anti-SARS-CoV-2 monoclonal antibodies (NIH, 2022b).

American Medical Association

The AMA released public health guidelines and recommendations concerning serological testing for SARS-CoV-2 antibodies on May 14, 2020. They list the limitations of antibody testing to include the potential for false-positive results, potential cross-reactivity, and lack of knowledge concerning relationship between antibody testing and immune status. The AMA recommends the following (AMA, 2020):

- "Use of serology tests should currently be limited to population-level seroprevalence study, evaluation of recovered individuals for convalescent plasma donations, and in other situations where they are used as part of a well-defined testing plan and in concert with other clinical information by physicians well-versed in interpretation of serology test results."
- "Serology tests should not be offered to individuals as a method of determining immune status."
- "Serology tests should not currently be used as the basis for any "immunity certificates," to inform decisions to return to work, or to otherwise inform physical distancing decisions. Doing so may put individuals, their household and their community at risk."
- "Serology tests should not be used as the sole basis of diagnosis of COVID-19 infection."

"Messaging on serological testing to medically underserved communities should explicitly take into consideration cultural and social features which may bear on their ability to make long-term choices on physical distancing and other COVID-19 precautions (AMA, 2020)."

Infectious Diseases Society of America

The Infectious Diseases Society of America (IDSA) on May 6, 2020, released their guidelines on the diagnosis of COVID-19. At this time, they focus solely on the use of targeted nucleic acid testing, such as RT-PCR, because "[a]t the time of this review, there was little evidence to inform use of serologic testing" (IDSA, 2020b). The IDSA convened a multidisciplinary panel of experts to review the research and literature on the available diagnostic testing for COVID-19. The panel used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology to assess the evidence of the studies and to make their recommendations. A primary recommendation implies that diagnostic testing and specimen collection devices are available whereas a contingency recommendation is made for situations where testing and/or personal protective equipment (PPE) are limited.

The panel made 17 recommendations concerning the use of nucleic acid testing as follows (IDSA, 2020b):

- They **strongly** recommend using a nucleic acid amplification test (NAAT), such as RT-PCR, in symptomatic patients even when clinical suspicion for COVID-19 is low.
- They suggest **(conditional recommendation)** using a nasopharyngeal, mid-turbinate, anterior nasal swab, saliva, or a combined anterior nasal/oropharyngeal swab rather than oropharyngeal swab or saliva sample for testing in symptomatic individuals suspected of COVID-19.
- They suggest **(conditional recommendation**) that either a patient or a healthcare provider can collect an anterior nasal or mid-turbinate sample in a symptomatic patient with upper respiratory tract infections or influenza-like illness suspected of COVID-19.
- They suggest **(conditional recommendation)** "initially obtaining an upper respiratory tract sample (e.g., nasopharyngeal swab) rather than a lower respiratory sample for SARS-CoV-2 RNA testing in hospitalized patients with suspected COVID-19 lower respiratory tract in infection.
    - If the initial upper respiratory sample result is negative, and the suspicion for disease remains high, the IDSA panel suggests collecting a lower respiratory tract sample (e.g., sputum, bronchoalveolar lavage fluid, tracheal aspirate) rather than collecting another upper respiratory sample."
- They suggest **(conditional recommendation)** performing only one test in a symptomatic individual and not repeat testing if low clinical suspicion of COVID-19.
- They suggest **(conditional recommendation**) repeat testing of an initial negative result in a symptomatic individual be performed only if there is an intermediate or high clinical suspicion of COVID-19.
- They suggest **(conditional recommendation)** "using either rapid RT-PCR or standard laboratory-based NAATs over rapid isothermal NAAT in symptomatic individuals suspected of having COVID-19)."
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals who are either known or suspected to have been exposed to COVID-19.
- They suggest against **(conditional recommendation)** RNA testing in asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with low prevalence. They consider a low prevalence rate to be less than 2% of the community.
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals with no known contact with COVID-19 who are being hospitalized in areas with high prevalence of the disease. They consider a high prevalence rate to be 10% or higher. The IDSA does note that if the prevalence rate is between 2% and 9% the decision to test should be dependent on the availability of testing resources.
- They **strongly** recommend RNA testing in immunocompromised asymptomatic individuals who are being admitted to the hospital regardless of exposure to COVID-19.
- They **strongly** recommend RNA testing (*versus* no testing) in asymptomatic individuals before immunosuppressive procedures, such as a hematopoietic stem cell (HSCT) or solid organ (SOT) transplant regardless of a known exposure to COVID-19.
- They make **NO** recommendations "for or against SARS-CoV-2 RNA testing before initiating immunosuppressive therapy in asymptomatic individuals with cancer," citing an evidence gap. This recommendation does not apply to candidates or recipients of HSCT or SOT.
- They make **NO** recommendations "for or against SARS-CoV-2 RNA testing before the initiation of immunosuppressive therapy in asymptomatic individuals with autoimmune disease," citing an evidence gap.

- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals without known exposure to COVID-19 who are undergoing major time-sensitive surgeries.
- They suggest against **(conditional recommendation)** RNA testing in asymptomatic individuals without known exposure to COVID-19 who are undergoing time-sensitive aerosol-generating procedures, such as a bronchoscopy, when PPE is available.
- They suggest **(conditional recommendation)** RNA testing in asymptomatic individuals without known exposure to COVID-19 who are undergoing time-sensitive aerosol-generating procedures when PPE is limited and testing is available. For this recommendation, the IDSA gives greater detail due to restrictions in availability of PPE. They also note that their recommendation does not address the need for repeat testing if patients require multiple procedures over time.

Besides the 17 recommendations, the IDSA panel also released their algorithm for SARS-CoV-2 Nucleic Acid Testing. This algorithm, as seen in **Figure 2**, separates individuals into symptomatic and asymptomatic groups. The IDSA notes that testing should be prioritized for symptomatic patients first. When resources are sufficient, then testing for selected asymptomatic individuals can be considered. Regardless, the preferred testing methodology is direct SARS-CoV-2 nucleic acid amplification testing, such as RT-PCR.

Figure 1. IDSA Algorithm for SARS-CoV-2 Nucleic Acid Testing

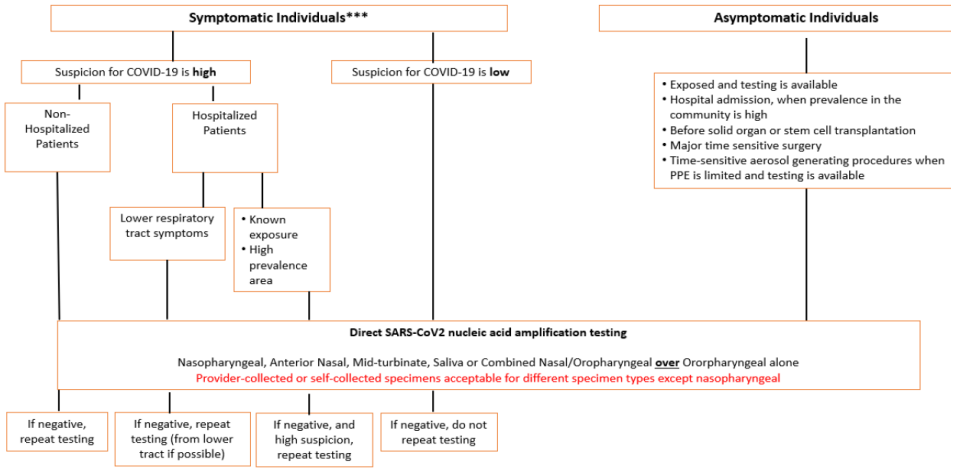

Figure 2: *IDSA Algorithm for SARS-CoV-2 Nucleic Acid Testing* (IDSA, 2020a). The Infectious Diseases Society of America (IDSA) released their algorithm for nucleic acid testing for COVID-19. According to the IDSA guidelines, testing priority should first be given to symptomatic patients; if resources are available, then testing asymptomatic individuals can be considered. Regardless, patients undergoing time-sensitive immunosuppressive procedures should be tested (IDSA, 2020b).

IDSA also published a guideline regarding serology testing on August 18, 2020. In it, they make the following recommendations:

- "The IDSA panel suggests against using serologic testing to diagnose SARS-CoV-2 infection during the first two weeks (14 days) following symptom onset (conditional recommendation, very low certainty of evidence)."
- "When SARS-CoV-2 infection requires laboratory confirmation for clinical or epidemiological purposes, the IDSA panel suggests testing for SARS-CoV-2 IgG or total antibody three to four weeks after symptom onset to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence)."
- The IDSA panel makes no recommendation either for or against using IgM antibodies to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence).
- "The IDSA panel suggests against using IgA antibodies to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence)."
- "The IDSA panel suggests against using IgM or IgG antibody combination tests to detect evidence of past SARS-CoV-2 infection (conditional recommendation, very low certainty of evidence)."
- "The IDSA panel suggests using IgG antibody to provide evidence of COVID-19 infection in symptomatic patients with a high clinical suspicion and repeatedly negative NAAT testing (weak recommendation, very low certainty of evidence)."
- "In pediatric patients with multisystem inflammatory syndrome, the IDSA panel suggests using both IgG antibody and NAAT to provide evidence of current or past COVID-19 infection (strong recommendation, very low certainty of evidence)."
- "The IDSA panel makes no recommendation for or against using capillary versus venous blood for serologic testing to detect SARS-CoV-2 antibodies (knowledge gap)."

IDSA also includes several comments on testing methodologies that are currently under evaluation. The methodologies named in this section of the guideline are "neutralizing antibody and cellular immune responses", detection of viral antigens aside from the S or N protein, and multi-test algorithms (IDSA, 2020c).

In the 2018 IDSA guidelines, released before the COVID-19 pandemic, IDSA notes, "Suspected cases of SARS coronavirus and MERS coronavirus require immediate notification to the laboratory. Guidance for testing can be found at [CDC websites for SARS and MERS]." For the four endemic human coronaviruses, they only state that they are associated with common cold "symptoms of rhinorrhea, congestion, sore throat, sneezing, and cough and may present with fever". They do note that for children with asthma or otitis media, these viruses can cause exacerbation of the conditions. IDSA notes, "Diagnostic tests include NAATs, which are now common in commercial respiratory panels." Within their table for the laboratory diagnosis of bronchiolitis, bronchitis, and pertussis, the IDSA lists possible diagnostic procedures for the detection of coronavirus to include NAAT, rapid antigen detection tests, and virus culture; however, they do not list one methodology as a preferred or recommended method over another. For the antigen testing, they do include a footnote stating, "Rapid antigen tests for respiratory virus detection lack sensitivity and depending upon the product, specificity" (Miller et al., 2018).

Occupational Safety and Health Administration

In the OSHA guidelines for employers, they classify jobs based on exposure risk to COVID-19 (lower, medium, high, or very high). In general, they recommend following CDC and local health department

guidelines. They do state that employers should not require documentation for employees to return to work since healthcare provider offices and medical facilities may be extremely busy during the crisis. For jobs classified at medium exposure risk, administrative controls do include the responsibility of the employer to "communicate the availability of medical screening or other worker health resources (e.g., on-site nurse; telemedicine services)." For jobs classified at high or very high exposure risk, administrative controls include that employers should "consider offering enhanced medical monitoring of workers during COVID-19 outbreaks" (OSHA, 2020). OSHA does not state what the term enhanced medical monitoring entails. This guideline is now considered an OSHA Archive Document, and is presented as "historical content, for research and review purposes only."

On June 10, 2021, OSHA updated its guidelines for "Protecting Workers: Guidance on Mitigating and Preventing the Spread of COVID-19 in the Workplace." The guidance was published to "help employers and workers not covered by the Occupational Safety and Health Administration's (OSHA's) COVID-19 Emergency Temporary Standard (ETS) to identify COVID-19 exposure risks to workers who are unvaccinated or otherwise at-risk even if they are fully vaccinated (e.g., if they are immunocompromised." (OSHA, 2021). The OSHA guideline has been modified to focus "only on protecting unvaccinated or otherwise at-risk, including if they are immunocompromised, and also [on] implement[ing] new guidance involving workers who are fully vaccinated but located in areas of substantial or high community transmission."

For implementing the most effective COVID-19 prevention programs, OSHA mentions employers should have measures "such as telework and flexible schedules, engineering controls (especially ventilation), administrative policies (e.g., vaccination policies), personal protective equipment (PPE), face coverings, physical distancing, and enhanced cleaning programs with a focus on high-touch surfaces." For protecting "unvaccinated or otherwise at-risk workers and mitigating the spread of COVID-19," employers should:

1. "Facilitate employees getting vaccinated. Employers should grant paid time off for employees to get vaccinated and recover from any side effects."
2. "Instruct any workers who are infected, unvaccinated workers who have had close contact with someone who tested positive for SARS-CoV-2, and all workers with COVID-19 symptoms to stay home from work to prevent or reduce the risk of transmission of the virus that causes COVID-19."
3. "Implement physical distancing in all communal work areas for unvaccinated and otherwise at-risk workers.
    a. Employers could also limit the number of unvaccinated or otherwise at-risk workers in one place at any given time, for example by implementing flexible worksites (e.g., telework); implementing flexible work hours (e.g., rotate or stagger shifts to limit the number of such workers in the workplace at the same time); delivering services remotely (e.g., phone, video, or web); or implementing flexible meeting and travel options, all for such workers.
    b. At fixed workstations where unvaccinated or otherwise at-risk workers are not able to remain at least 6 feet away from other people, transparent shields or other solid barriers can separate these workers from other people."
4. "Provide workers with face coverings or surgical masks, as appropriate, unless their work task requires a respirator or other PPE.
    a. Employers should provide face coverings to unvaccinated and otherwise at-risk workers at no

cost (and make replacements available to workers when they request them).

b. Unless otherwise provided by federal, state, or local requirements, unvaccinated workers who are outdoors may opt not to wear face coverings unless they are at-risk, for example, if they are immunocompromised.

c. When an employer determines that PPE is necessary to protect unvaccinated and otherwise at-risk workers from exposure to COVID-19, the employer must provide PPE in accordance with relevant mandatory OSHA standards and should consider providing PPE in accordance with other industry-specific guidance."

5. "Educate and train workers on your COVID-19 policies and procedures using accessible formats and in languages they understand.
   a. Training should be directed at employees, contractors, and any other individuals on site, as appropriate, and should include:
      i. Basic facts about COVID-19, including how it is spread and the importance of physical distancing (including remote work), ventilation, vaccination, use of face coverings, and hand hygiene.
      ii. Workplace policies and procedures implemented to protect workers from COVID-19 hazards.
   b. In addition, ensure that workers understand their rights to a safe and healthful work environment, whom to contact with questions or concerns about workplace safety and health, and their right to raise workplace safety and health concerns free from retaliation."
6. "Suggest or require that unvaccinated customers, visitors, or guests wear face coverings, especially in public-facing workplaces such as retail establishments, and that all customers, visitors, or guests wear face coverings in public, indoor settings in areas of substantial or high transmission."
7. "Maintain ventilation systems."
8. "Perform routine cleaning and disinfection.
   a. If someone who has been in the facility within 24 hours is suspected of having or confirmed to have COVID-19, follow the CDC cleaning and disinfection recommendations."
9. "Record and report COVID-19 infections and deaths.
   a. Under mandatory OSHA rules in 29 CFR part 1904, employers are required to record work-related cases of COVID-19 illness on OSHA's Form 300 logs if the following requirements are met: (1) the case is a confirmed case of COVID-19; (2) the case is work-related (as defined by 29 CFR 1904.5); and (3) the case involves one or more relevant recording criteria (set forth in 29 CFR 1904.7) (e.g., medical treatment, days away from work). Employers must follow the requirements in 29 CFR part 1904 when reporting COVID-19 fatalities and hospitalizations to OSHA. More information is available on OSHA's website. Employers should also report outbreaks to local health departments as required and support their contact tracing efforts."
10. "Implement protections from retaliation and setting up an anonymous process for workers to voice concerns about COVID-19-related hazards."
11. "Follow other applicable mandatory OSHA standards.
    a. All of OSHA's standards that apply to protecting workers from infection remain in place. These mandatory OSHA standards include: requirements for PPE (29 CFR part 1910, Subpart I (e.g., 1910.132 and 133)), respiratory protection (29 CFR 1910.134), sanitation (29 CFR 1910.141), protection from bloodborne pathogens: (29 CFR 1910.1030), and OSHA's requirements for employee access to medical and exposure records (29 CFR 1910.1020). Many healthcare

workplaces will be covered by the mandatory OSHA COVID-19 Emergency Temporary Standard"(OSHA, 2021).

Within the workplace, OSHA recommends "maintaining ventilation systems, implementing physical distancing, and properly using face coverings (or other personal protective equipment (PPE) and respiratory protection such as N95 respirators when appropriate) and proper cleaning" (OSHA, 2021).

American Association for Clinical Chemistry (AACC)

The AACC released a set of recommendations for "implementing and interpreting SARS-CoV-2 EUA and LDT [laboratory developed test] serologic testing in clinical laboratories." Serologic testing is currently only used for serum, plasma, and "less frequently, whole-blood or dried blood spots," but not for other sample types, like saliva and cerebrospinal fluid. Serologic testing is "not recommended as the primary approach for diagnosis of SARS-CoV-2 infection." For the recommended use of serologic testing, the AACC stated the following:

- "Serologic testing may be offered as an approach to support diagnosis of COVID -19 illness in symptomatic patients and late phase negative molecular testing or for patients presenting with late complications such as multisystem inflammatory syndrome in children (MIS -C).
- Serologic testing can help identify people who may have been infected with or have recovered from the SARS -CoV -2 infection.
- Serologic testing can be used to screen potential convalescent plasma donors and in the manufacture of convalescent plasma.
- Serologic testing can be used for epidemiology and seroprevalence studies.
- Serologic testing can be used for vaccine response and efficacy studies."

Regarding serologic testing limitations, the AACC stated the following:

- "False positive results may occur.
- Negative results do not preclude acute SARS CoV-2 infection or viral shedding.
- Serologic tests may not differentiate between natural infection and vaccine response.
- Serologic results should not be used for
  - Determining individual protective immunity
  - Return to work decisions
  - Cohorting individuals in congregate settings
  - Assessment of convalescent plasma recipients
  - Use of Personal Protective Equipment
  - Placement of high-risk job functions" (Zhang et al., 2021)

European Centre for Disease Prevention and Control

The ECDC in their guidance for laboratory support in the EU/EEA recommends using WHO-recommended testing strategies for the diagnosis and confirmation of COVID-19 (ECDC, 2022).

In the ECDC's guideline titled "COVID-19 testing strategies and objectives", the ECDC recommends performing laboratory testing in accordance with the WHO case definition. The following populations

should be tested (ECDC, 2020):

- "Ideally, all people with COVID-19 symptoms should be tested as soon as possible after symptom onset. This requires easy access to testing for all, including non-residents. Test result turnaround time should be minimized, people testing positive should isolate and timely contact tracing should be carried out, ensuring that all close contacts are tested, irrespective of symptoms.
- All patients with acute respiratory symptoms in hospitals and in other healthcare settings, and all specimens from sentinel primary care surveillance should be tested for both SARS-CoV-2 and influenza during the influenza season to monitor incidence and trends over time.
- Healthcare and social care settings require intensive testing when there is documented community transmission. Periodic and comprehensive testing of all staff and residents/patients is recommended to prevent nosocomial transmission. Furthermore, all patients/residents should be tested upon or just prior to admission.
- Clusters or outbreaks may occur in certain settings, such as workplaces, educational facilities, prisons, and migrant detention centres. Testing policies and systems should be in place for rapid detection and control to protect the relevant populations in these settings and to protect the community from amplified transmission.
- Countries experiencing high SARS-CoV-2 transmission in a local community should consider testing the whole population of the affected area. This would enable identification of infectious COVID-19 cases and allow for their prompt isolation to interrupt chains of transmission. Depending on the epidemiological situation, size and population density of the affected area, such an approach could be less disruptive for society than having to introduce and ensure compliance with more stringent public health measures.
- To prevent re-introduction, countries or subnational areas that achieved sustained control of the circulation of SARS-CoV-2 should, in addition to quarantine measures, consider targeted testing and follow-up of individuals coming from other areas within the same country, or from other countries that have not yet achieved sustained control of the virus" (ECDC, 2020).

Finally, ECDC notes that "Genomic surveillance of SARS-CoV-2 is essential to detect, monitor and assess virus variants that can result in increased transmissibility, disease severity, or have other adverse effects on public health and social control measures. Obtaining timely and accurate information on the emergence and circulation of variants of concern (VOCs) and variants of interest (VOIs) requires robust surveillance systems, including integrated genome sequencing with a well-defined sampling and sequencing strategy to ensure representativeness and reliability of findings" (ECDC, 2020, 2021).

American Academy of Pediatrics

The AAP lists the most common scenarios for testing as inclusive of: symptomatic patients, patients who are asymptomatic but had exposure to a person with confirmed or probable COVID-19 infection, and patients who required screening as part of local public health, school, or workplace requirement. The AAP notes that a person's vaccination status may be a factor in decision-making concerning the need for screening.

Additionally, the AAP says that for patients who have symptoms, both NAATs (such as PCR testing) and antigen tests can be used. A positive result indicates a SARS-CoV-2 infection on either PCR or antigen

diagnostics. That said, for a patient with a negative antigen result, a provider may repeat the antigen test at 48 hours per FDA guidance.

For purposes of testing symptomatic children who have recently had confirmed infections within 3 months, the AAP says providers should consider the possibility of a false-positive result. Especially using PCR tests and other NAAT tests, as these may remain positive from deposited viral genetic material for several months after an active infection. The AAP notes, "In a child with known exposure and compatible symptoms, there may be situations in which it is reasonable to retest within the 90-day window. If testing is performed within that window, antigen testing is generally preferable to NAATs because of the potential for positive NAAT results attributable to prior infection" (AAP, 2022a).

Further, the AAP previously stated in 2020-2021 guidance that antibody (serologic) tests "can provide evidence of previous infection with SARS-CoV-2 but are not useful for the diagnosis of acute infection. A positive antibody test result does not prove that a patient has protection against SARS-CoV-2, although the FDA and vaccine companies use serologic testing as a marker for immunogenicity and protection from SARS-CoV-2 infection. Thus, these tests should not be used to make decisions on grouping people in classrooms or other facilities at this time, and individuals with positive antibody tests should continue to adhere to guidelines about masking, social distancing, and other preventive measures" (AAP, 2022a).

The AAP has also included some comments and discussion on Multisystem Inflammatory Syndrome in Children (MIS-C). MIS-C has been observed to have some association with COVID-19, and patients with this syndrome have been observed to test positive "far more often" for past SARS-CoV-2 infection (i.e. antibody testing) than acute infection (RT-PCR or antigen test). The CDC defines an MIS-C case by the following criteria:

- "An individual aged < 21 years presenting with:
  - fever (> 38.°C for ≥ 24 hours, or report of subjective fever lasting ≥ 24 hours),
  - laboratory evidence of inflammation (including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, D-dimer, ferritin, LDH, or IL-6, elevated neutrophils, reduced lymphocytes, low albumin),
  - evidence of clinically severe illness requiring hospitalization,
  - with multisystem (≥ 2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND
- No alternative plausible diagnoses; AND
- Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms.

The CDC delineates a testing algorithm for MIS-C as follows:

- "Evaluate a child who presents with a persistent fever (≥ 3 days) who is moderately to severely ill with clinical signs of organ dysfunction."
- "Early consultation and coordination with the nearest pediatric infectious disease and rheumatology specialist and pediatric referral center for optimal testing and management should be considered.."

- "Laboratory screening for systemic inflammation may be considered and initial lab screenings may include complete blood cell count (CBC) with differential, urine analysis, ESR, and CRP, with the addition of ferritin, LDH, comprehensive metabolic panel, pro-BNP, troponin, and fibrinogen depending on initial clinical suspicion and/or evidence of inflammation on initial lab screening."
- "Any child sick enough to warrant admission for fever, abdominal pain, diarrhea, and/or organ dysfunction in whom MIS-C is suspected should be cared for in a hospital with tertiary pediatric/cardiac intensive care units.
    - Chest radiograph, EKG, and troponin. If any of these or physical examination is abnormal, then consult with pediatric cardiology and consider additional diagnostic testing for myocardial injury (echocardiogram and/or cardiac MRI).
    - Expanded laboratory tests including pro-BNP, triglycerides, creatine kinase, amylase, blood and urine culture, D-dimer, prothrombin time/partial thromboplastin time (PT/PTT), INR, CRP, ferritin, LDH, comprehensive metabolic panel, and fibrinogen, if not already conducted.
    - In all cases, COVID-19 testing should be performed with RT-PCR assay and serologic testing. Later serology may be needed if all are negative initially. Serologic tests must be sent prior to administration of intravenous immunoglobulin (IVIG)." (AAP, 2022b).

American College of Rheumatology

The ACR published guidance regarding MIS-C associated with COVID-19. In it, they list SARS-CoV-2 IgG, IgM, and IgA as part of the diagnostic pathway for MIS-C (Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, MB, et al., 2020).

In a December 5, 2020 update of the above guidelines, the ACR states that ESR, CRP, and testing for SARS-CoV-2 (by PCR or serology) should be considered a "tier 1" (first-line evaluation) for MIS-C (Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, Son, et al., 2020).

In a February 3, 2022 update of the above guideline, the ACR added new information concerning immunomodulatory treatment in MIS-C, hyperinflammation in COVID-19, as well as statements on thrombotic risk and anticoagulation in MIS-C (Henderson et al., 2022).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA issued an "Immediately in Effect Guidance on policy for diagnostics testing in laboratories certified to perform high complexity testing under CLIA prior to Emergency Use Authorization for Coronavirus Disease-2019 during the public health emergency" in February 2020 (FDA, 2022c). This policy was updated on 05/11/2020 to state that the "policy is intended to remain in effect only for the duration of the public health emergency related to COVID-19 declared by the Secretary of Health and Human Services (HHS) on January 31, 2020, effective January 27, 2020, including any renewals made by the HHS Secretary in accordance with section 319(a)(2) of the Public Health Service Act (PHS Act) (FDA, 2022e)." As of October 15, 2021, the FDA had issued 418 different EUAs for COVID-19 testing for either

in vitro diagnostic products (which includes testing such as point-of-care tests, antibody testing, and antigen testing) or high complexity molecular-based laboratory developed tests (FDA, 2021a).

Moreover, within the HR 748, passed as the CARES Act (or Coronavirus Aid, Relief, and Economic Security Act) as public law 116-136 on March 27, 2020, there are sections concerning coverage and pricing of diagnostic testing for COVID-19 (US, 2020).

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
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (e.g., reagent strip)
86328	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (e.g., reagent strip); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
86408	Neutralizing antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID19]); screen
86409	Neutralizing antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID19]); titer
86413	Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) (Coronavirus disease [COVID-19]) antibody, quantitative
86769	Antibody; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
86790	Antibody: virus, not elsewhere specified
87426	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; severe acute respiratory syndrome coronavirus (e.g., SARS-CoV, SARS-CoV-2 [COVID-19])
87428	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; severe acute respiratory syndrome coronavirus (e.g., SARS-CoV, SARS-CoV-2 [COVID-19]) and influenza virus types A and B
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
87635	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87811	Infectious agent antigen detection by immunoassay with direct optical (i.e., visual) observation; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
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
0202U	Infectious disease (bacterial or viral respiratory tract infection), pathogen specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected Proprietary test: BioFire® Respiratory Panel 2.1 (RP2.1) Lab/Manufacturer: BioFire®Diagnostics, LLC
0223U	Infectious disease (bacterial or viral respiratory tract infection), pathogen-specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected Proprietary test: QIAstat-Dx Respiratory SARS CoV-2 Panel Lab/Manufacturer: QIAGEN Sciences

0224U	Antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), includes titer(s), when performed Proprietary test: COVID-19 Antibody Test Lab/Manufacturer: Mt Sinai, Mount Sinai Laboratory
0225U	Infectious disease (bacterial or viral respiratory tract infection) pathogen-specific DNA and RNA, 21 targets, including severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected Proprietary test: ePlex® Respiratory Pathogen Panel 2 Lab/Manufacturer: GenMark Dx/GenMark Diagnostics, Inc
0226U	Surrogate viral neutralization test (sVNT), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), ELISA, plasma, serum Proprietary test: Tru-Immune™ Lab/Manufacturer: Ethos Laboratories/GenScript® USA Inc
C9803	Hospital outpatient clinic visit specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19]), any specimen source
G2023	Specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19]), any specimen source
G2024	Specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19]) from an individual in a SNF or by a laboratory on behalf of a HHA, any specimen source
U0001	CDC Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel
U0002	Non-CDC laboratory test for 2019-nCoV (COVID-19), any method
U0003	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique, making use of high throughput technologies as described by CMS-2020-01-R
U0004	2019-nCoV Coronavirus, SARS-CoV-2/2019-nCoV (COVID-19), any technique, multiple types or subtypes (includes all targets), non-CDC, making use of high throughput technologies as described by CMS-2020-01-R
U0005	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique, CDC or non-CDC, making use of high throughput technologies, completed within two calendar days from date and time of specimen collection. (List separately in addition to either HCPCS code U0003 or U0004)

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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Diagnosis of Idiopathic Environmental Intolerance

Policy #: AHS – G2056	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/26/22 (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

Idiopathic environmental intolerance (IEI), formerly called multiple chemical sensitivity (MCS), is a subjective condition characterized by recurrent, nonspecific symptoms attributed to low levels of chemical, biologic, or physical agents in the absence of consistent objective diagnostic physical findings or laboratory tests that define an illness (AAAAI, 1999; ACOEM, 1999; Black & Temple, 2019).

II. Related Policies

Policy Number	Policy Title
AHS-G2031	Allergen Testing
AHS-G2099	Intracellular Micronutrient Analysis

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.

Diagnosis of Idiopathic Environmental Intolerance, 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.

1. In all circumstances, laboratory tests designed to confirm the diagnosis of idiopathic environmental intolerance **DO NOT MEET COVERAGE CRITERIA.**

2. In all circumstances, the screening of blood, saliva, serum, plasma, urine, and/or stool samples for volatile solvents, organic acids, and organophosphates **DOES NOT MEET COVERAGE CRITERIA**:

3. In all circumstances, profiling of phthalates and parabens using a blood, serum, plasma, saliva, urine, and/or stool sample **DOES NOT MEET COVERAGE CRITERIA.**

4. For asymptomatic individuals, profiling of chlorinated pesticides, including DDE and DDT, using a blood, serum, plasma, saliva, urine, and/or tool samples **DOES NOT MEET COVERAGE CRITERIA.**

5. In asymptomatic individuals and/or during general encounters without abnormal findings, testing of blood, serum, plasma, saliva, urine, and/or stool samples for carnitine sufficiency, oxidative stress and antioxidant sufficiency, detoxification adequacy, methylation sufficiency status, lipoic acid and CoQ10 sufficiency, and/or intestinal hyperpermeability **DO NOT MEET COVERAGE CRITERIA.**

6. In asymptomatic individuals and/or during general encounters without abnormal findings, testing of blood, serum, plasma, saliva, urine, and/or stool samples for vitamin sufficiency, mineral sufficiency, and/or nutritional analysis **DO NOT MEET COVERAGE CRITERIA** in asymptomatic individuals and/or during general encounters without abnormal findings.

7. The use of a breath hydrogen and/or breath methane test to assess or diagnose the following conditions **DOES NOT MEET COVERAGE CRITERIA**:

    a. Idiopathic environmental intolerance
    b. Food allergies and sensitivities
    c. Carbohydrate sensitivity or intolerance,
    d. Digestive disorders
    e. Constipation, diarrhea, or flatulence
    f. Neurological/neuromuscular disorders,
    g. Rosacea
    h. Obesity
    i. As part of a wellness visit and/or general encounter without abnormal findings

8. In asymptomatic individuals and/or during general encounters without abnormal findings, testing of blood, serum, urine, cerebrospinal fluid, fingernails, hair, and/or stool sample for metals, **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Patients with idiopathic environmental intolerance (IEI) typically report sensitivity to multiple, chemically unrelated substances and become ill due to a wide range of nonspecific symptoms when exposed. Symptoms may include anxiety, shortness of breath, chest pain, and more. Psychiatric disorders may also be at the core of the IEI patient (D. Black & Temple, 2021). The mean age of patients reporting IEI is between 30 and 40 years, women are diagnosed more than men, and individuals who are married are significantly more likely to be diagnosed with IEI than those who are not (Black & Temple, 2021). IEI also occurs in 40% of people with chronic fatigue syndrome and in 16% of people with fibromyalgia (Black et al., 2020).

The symptoms of IEI are nonspecific, ambiguous and common in the general population. There is no characteristic set of symptoms and ultimately no major differences between patients self-reporting IEI and those that do not. Virtually any symptom can be considered a symptom of IEI (Black & Temple, 2021). Within the definition of multiple chemical sensitivity (MCS), identified symptoms included "asthmatic-like, skin irritation, dermatitis, migraine, dysuria, dyspepsia, symptoms of supposed sensitization to food, persistent arthromial pain, vertigo, vestibular impairment," with 80% of patients experiencing "asthenia, arthromial pain, dyspepsia, coriza, eructation, chest pain, insomnia" (Quarato et al., 2020). The classification of IEI as a distinct medical disorder is also in question, as a lack of reliable case reports, lack of consistent findings or laboratory results, and reliance on surveys or self-reporting all cloud the condition and understanding of this disorder (Black & Temple, 2021).

Recently, many articles have been published suggesting a relationship between electromagnetic fields and IEI. Electromagnetic fields may include radiofrequencies from telecommunication devices (Eltiti et al., 2018; Huang et al. 2018), Wi-Fi and base stations (ANSES, 2018). For an unknown reason, these individuals claim to react to the exposure of certain electromagnetic triggers that most people can tolerate without issues; these triggers are below established toxicological and hazardous thresholds. ANSES (2018) researched the relationship between electric field exposure and IEI symptoms and stated that "either the symptoms experienced by EHS [electromagnetic hypersensitivity] individuals are not caused by exposure to electromagnetic fields and there are no quantifiable biological and/or physiological abnormalities when they are exposed to electromagnetic fields (assumption 1) or the absence of results is due to the methodological limitations of the provocation studies (subject selection, sample size, exposure type, etc.) (assumption 2)." These findings were corroborated by Schmiedchen et al. (2019), who, in their systematic review of articles pertaining to EHS, stated, "limitations in design, conduct and analysis could therefore have given rise to either false positive for false negative results," and that the "nocebo effect or medical/mental disorders may explain the complaints in many individuals." Characteristic symptoms of EHS include sleep and circadian rhythm disorders, migraines and headaches, hypersensitivity, and other related syndromes and disorders such as fibromyalgia, tinnitus and MCS (ANSES, 2018).

Tests such as elimination diets, food challenges, and provocation-neutralization tests have been used to

test for food or chemical sensitivities. Immunological tests or tests measuring the amount of various chemicals in body tissues have also been performed (Black & Temple, 2021). In fact, testing for a wide range of autoantibodies is generally discouraged, as "pretest probability is low, and false-positive results are far more likely than true-positive results; a weakly positive ANA [antinuclear antibodies] is present in about 20% of the population" (Black et al., 2020). However, these assessments are typically not rigorous enough to provide strong evidence; for example, these tests are often not performed blinded or with placebo controls. No unusual laboratory findings have been reliably linked to IEI (Black & Temple, 2021). Due to the vast amount of causes, symptoms, responses, and general heterogeneity of this condition, it may be very difficult to provide a scientifically valid or useful test. Worse, testing may even exacerbate or increase the number of symptoms of a patient. Physicians should use caution in testing for reassurance of patients as negative findings may increase anxiety instead (Barsky & Borus, 1999; Black & Temple, 2021).

Proprietary Testing

Due to the number of symptoms that may be considered part of IEI, there are a corresponding amount of tests performed. These tests are generally unnecessary as the condition itself is far too ambiguous to reliably test for and any test can be ordered under the guise of IEI. For example, assessment of factors such as elastase, stool culturing, or fat differentiation may all be done for the sake of IEI treatment. These tests may have legitimate medical purposes (for instance a stool culture may be useful for numerous conditions) but their use for IEI is essentially none, as IEI itself carries no reliable characteristics to test for. Other tests that evaluate a tangentially relevant analyte, such as micronutrient panels or a lactose intolerance breath test, may be done for IEI's sake as well. Since virtually any symptom or sign can be called IEI, these tests are sometimes ordered for nonspecific or subjective symptoms such as fatigue or pain. However, these tests cannot provide any useful results because of the dubious nature of IEI itself.

Another commonly used test for IEI are panels that test multiple factors in one. For example, the Triad Bloodspot Profile offered by Genova Diagnostics measures organic acid levels, "the level of IgG4 reactions for 30 common foods," and "essential amino acid imbalances" (Genova, 2021d). Genova offers several similar panels, such as the Organix Comprehensive Profile (which tests 46 analytes for subjective symptoms such as depression, weight issues and chemical sensitivities) (Genova, 2021c), the NutrEval FMV (which tests 118 analytes for symptoms such as fatigue, weight issues, and sports fitness optimization) (Genova, 2021a) and the Allergix IgG4 Food Antibodies (which tests 90 foods for sensitivity). Genova Diagnostics also offers the GI Effects Profile (advanced stool tests for the management of GI health), a full line of allergy testing and assessment tests (measuring IgG and IgE food antibodies, inhalants, molds and spices), the Ion Profile (which evaluates various types of organic, amino and fatty acids as well as nutrient and toxic elements), the CDSA 2.0 Profile with Parasitology (evaluates the microbiome, digestion and absorption), and SIBO Profile tests (breath tests which measure methane gases and exhaled hydrogen) (Genova, 2020).

An evaluation of symptoms of IEI patients includes a history, physical examination, and laboratory tests (complete blood count, serum electrolytes and glucose, urine analysis) with further testing guided by reported symptoms. An occupational or environmental history is also useful as patients typically report problems from chemical exposure (Black & Temple, 2021). A questionnaire such as the "Environmental Exposure and Sensitivity Intolerance" (EESI) may be used for an initial screening (Rossi & Pitidis, 2018). A psychiatric history is also recommended as psychiatric disorders are often co-morbid with IEI. A screening questionnaire such as the Patient Health Questionnaire (PHQ-9) can be used to identify psychiatric conditions in an IEI patient (Black & Temple, 2021; Gilbody et al., 2007).

Micronutrients are the essential vitamins and minerals required by the body for proper functioning. Panels have been developed which evaluate intracellular levels of essential vitamins and minerals. These panels may also be used on IEI patients. This may help to identify nutritional deficiencies in otherwise healthy patients or in patients suffering from some type of disease. SpectraCell Laboratories have developed the Micronutrient Test Panel, which is able to measure 31 vitamins, minerals, metabolites, amino acids, fatty acids and antioxidants; this test also measures how these micronutrients affect cellular functioning in an individual (SpectaCell, 2021). SpectraCell Laboratories have also developed the SPECTROX™, claiming it measures total antioxidant function in an individual, reporting on the repair mechanisms and net ability of each individual's cells (SpectraCell, 2008). As noted above, Genova Diagnostics has developed the NutrEval FMV that measures 118 markers, including amino acids, fatty acids and organic acids (Genova, 2021a). ONE (Optimal Nutritional Evaluation) FMV, also by Genova Diagnostics, is a urine-based nutritional test which assesses "the functional need for antioxidants, B-vitamins, minerals, digestive support and amino acids" (Genova, 2021b). The company notes that the ONE FMV test may be used for patients with mood disorders, fatigue, digestive issues, weight problems, general health, dietary guidance and fitness. Another nutrient panel blood test, developed by Life Extension, measures vitamin B12, folate, vitamin D 25-hydroxy, vitamin A, vitamin C, selenium, zinc, CoQ10 (coenzyme Q10) and magnesium (LifeExtension, 2020). Finally, Vibrant America provides a test which measures approximately 40 intracellular and extracellular vitamins, minerals, fatty acids, amino acids and antioxidants (Vibrant, 2017).

Clinical Utility and Validity

Very little information suggests that the intracellular micronutrient analysis assists with positive health outcomes. Houston (2013) published an article on the role of vitamins, minerals and overall nutrition in the prevention and treatment of hypertension. This article reviewed hypertension-related clinical trials that include information on the "efficacy of nutrition, weight loss, exercise, and nutritional supplements, vitamins, minerals, and antioxidants" (Houston, 2013). Approximately 3338 patients were treated with micronutrient testing over a five-year period, with 20% of these patients exhibiting abnormally high blood pressure. After six months, 62% of the hypertensive patients reached lower blood pressure goals. Hence, the author states that the diagnosis and treatment of various nutritional deficiencies can decrease the number of cardiac events as well as reduce blood pressure and improve vascular biology. However, data for the control group not treated with micronutrients was not provided for comparison.

Another technique that has been used to assess nutritional status is the measurement of the hepatic proteins prealbumin and albumin. However, it seems that a physical examination has evolved as the main technique to diagnose malnutrition in a clinical setting. “The current consensus is that laboratory markers are not reliable by themselves but could be used as a complement to a thorough physical examination” in a malnutrition diagnosis (Bharadwaj et al., 2016). The Academy of Nutrition and Dietetics also do not accept albumin and prealbumin as a diagnostic tool for malnutrition and state that “There is no laboratory test that is both sensitive to and specific for protein-calorie malnutrition” (AND, 2017).

IEI patients may also report bowel irritability. Small intestinal bacterial overgrowth (SIBO) occurs when excessive aerobic and anaerobic bacteria colonize the small bowel. These bacteria are typically found in the colon and can cause chronic diarrhea and malabsorption if they colonize the small bowel (Pimentel, 2022). SIBO may be diagnosed by a breath test. However, a validated gold standard method for diagnosing SIBO has not been indicated (Rezaie et al., 2017). The SIBO breath test uses carbohydrates in a simple, non-invasive and widely available testing method. A carbohydrate substrate (such as lactulose or glucose) is administered to the patient, which leads to the production of an analyte such as hydrogen or methane. “In individuals without SIBO, the administration of lactulose results in a single peak in breath hydrogen/methane within two to three hours due to the metabolism of lactulose by colonic flora. In patients with SIBO, administration of lactulose results in an early peak in breath hydrogen/methane levels due to metabolism by small bowel bacteria” (Pimentel, 2019). As noted above, Genova Diagnostics has developed the SIBO Profile test which is a two or three hour breath test that measures methane gases and exhaled hydrogen (Genova, 2020). This test requires the patient to ingest a lactulose solution.

Bratten et al. (2008) completed a study with 224 patients with irritable bowel syndrome (IBS) and 40 controls. A lactulose breath test (LBT) was used to measure methane and hydrogen production to identify patients with IBS. Results showed that “The majority of patients with IBS and healthy subjects meet criteria for an "abnormal" LBT using previously published test criteria, and groups are not discriminated using this diagnostic method” (Bratten et al., 2008). The authors then questioned the utility of an LBT to diagnose IBS as the testing did not discriminate between IBS patients and healthy controls. A more recent study by Ghoshal, Srivastava, Ghoshal, and Misra (2014) evaluated 80 patients with IBS for SIBO. Culture had previously diagnosed 15/80 patients with SIBO. Both lactulose and glucose hydrogen breath tests (LHBT and GHBT, respectively) were used to measure SIBO. The authors conclude that “The specificity of GHBT was 100%, but the sensitivity of this test and the diagnostic performances of LHBT and breath methane were all very poor” (Ghoshal et al., 2014).

Speck and Witthöft (2022) included 410 patients in a cross-sectional study design to investigate the relationship between IEI symptoms associated with chemicals and schizotypy spectrum. They found that “schizotypal traits were found to be significantly positively associated with [modern health worries], [chemical odor sensitivity] …, and showed significant positive associations with hallucination proneness.

Magical thinking was found to exhibit a significant positive relationship with both [modern health worries] and [chemical odor sensitivity].” This demonstrates how the principles surrounding IEI may need to consider associated psychiatric differential diagnoses to properly evaluate symptoms and testing. Finding that patients have symptoms of chemical odor sensitivity and modern health worries can also conversely encourage further insight into the mental wellness of a patient.

Madigan et al. (2022) investigated the relationship between SIBO caused by *Archaea* and certain clinical symptoms. *Archaea* are anaerobic bacteria that produce methane specifically. Through a retrospective cross-sectional study, the researchers used glucose breath tests conducted for SIBO to correlate the bacteria to their phenotypic manifestations. From 1461 patients, they found that 33.1% were SIBO positive, with 38.8% producing only methane, 11.4% producing both methane and hydrogen, and 49.8% with hydrogen only producing organisms. Methane-producing SIBO patients had an increased odds of experiencing constipation and gassiness in comparison to SIBO(-) patients. On the other hand, hydrogen-producing SIBO patients had several “significant factors”: “vitamin B12 deficiency (odds ratio, 1.44; CI, 1.01–2.06; P = .046), [Roux-en-Y Bypass] (odds ratio, 2.14; CI, 1.09–4.18; P = .027), cholecystectomy(odds ratio, 1.42; CI, 1.06–1.91; P = .020), , and diabetes (odds ratio, 1.59; CI, 1.13–2.24; P = .008).” However, when comparing methane-producing SIBO versus hydrogen-producing SIBO patients, “vitamin B12 deficiency was the only factor that reached significant (OR 0.57; CI, 0.34-0.97; P = 0.038), indicating that [methane-producing SIBO] patients were almost half as likely to report cobalamin deficiency.” This study demonstrated the implications of varying gas producing organisms in SIBO and the clinical symptoms that can affect treatment and prognosis, solely by extrapolating data from breath tests (Madigan et al., 2022).

Rangan et al. (2022) conducted a review to investigate the clinical utility and drawbacks of SIBO breath testing. They identified that the “variability in oral-cecal transit time” was the biggest limitation in breath testing, and that it greatly contributed to common false-positive test results. This theoretically results from lactulose fermentation by normal colonic flora versus invasive microbial flora. In comparing the specificity and sensitivity for lactulose breath testing versus glucose breath testing, it was found that the former had a sensitivity of 42.0% and specificity of 70.6%, whereas the latter had a sensitivity of 54.5% and a specificity of 83.2%. However, those with a positive lactulose breath test result were more likely to respond to rifaximin therapy, thereby implying greater clinical utility. Despite the controversies in the substrates for testing, the researchers state that “notably, however, clinical symptoms have also been shown to be nonspecific for diagnosing SIBO, and thus breath testing remains a useful diagnostic tool in managing those patients with compatible symptoms and an absence of another diagnosis on endoscopy or imaging, particularly if there are other underlying conditions that could predispose to SIBO” (Rangan et al., 2022).

Bushyhead and Quigley (2022) corroborates the technical difficulties and clinical utility of SIBO breath testing discussed in the two studies mentioned above. In their review, they state that breath testing is less invasive and inexpensive relative to small bowel culture-based diagnoses. However, there is no solidified association between methanogenic overgrowth and gastrointestinal symptoms like constipation, as the “positive breath test for methane may be due to methane production by resident

anaerobic colonic methanogens rather than small bowel flora." They also concur on the idea that "an important factor that may confound the interpretation of lactulose breath tests… is orocecal transit time…It is also possible that glucose malabsorption, which may be more prevalent than previously considered, could lead to a positive glucose breath test… Prior upper GI surgery could also contribute to accelerated orocecal transit of glucose; conversely, those with constipation and preformed gas can confound more test results." The variability and contamination limit the diagnostic utility of breath testing in the setting of SIBO (Bushyhead & Quigley, 2022).

V. Guidelines and Recommendations

Due to the dubious nature of this condition, several prominent medical studies have regarded this condition with suspicion. In 1992, the American Medical Association stated that multiple chemical sensitivity (now IEI) should not be recognized as a syndrome until accurate, reproducible, and well-controlled studies can be done (AMA, 1992). Other societies such as the American College of Physicians and the American Academy of Allergy and Immunology hold similar views (AAAAI, 1986; ACP, 1989).

American Academy of Allergy, Asthma and Immunology (AAAAI)

In 2006, AAAAI referenced IEI in their position statement on the medical effects of mold stating that testing many nonvalidated immune based tests, as had been done to suggest an immunologic basis for IEI (MCS), is expensive, not useful or valid, and should be discouraged (Bush et al., 2006).

American College of Occupational and Environmental Medicine (ACOEM)

In 1999, the ACOEM published a position statement that stated there have been no consistent physical findings or laboratory abnormalities in IEI (then called MCS) patients and recommended that a generalized clinical approach, such as establishing a therapeutic alliance and avoiding unnecessary tests, would be useful in the management of other nonspecific medical syndromes (ACOEM, 1999).

French Agency for Food, Environmental and Occupational Health & Safety (ANSES) Appraisal-Collective Expertise Report

An ANSES expert committee published an opinion piece regarding the expert appraisal on EHS or IEI due to electromagnetic fields. This committee did not find any conclusive results regarding IEI and therefore does not recommend any specific testing methods for this ailment, other than the psychological testing of patients.

Consensus Document (1999)

An international document, created by 89 clinicians and researchers with broad experience in the field, aimed to establish consensus criteria for MCS. The recognition criteria of MCS set forth by this expert panel are as follows:

- Chronic condition
- Reproducible symptoms with repeated chemical exposure
- Low exposure levels cause syndrome to occur
- Removal of offending agents cause symptoms to subside

The 19999 Consensus Document is the most widely used criteria for recognition of MCS (Martini et al., 2013).

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

The NASPGHAN and ESPGHAN have stated that "Clinicians should familiarize themselves with the limitations of nutritional biomarkers in the context of chronic liver disease" but do not give specific recommendations regarding nutritional laboratory testing (Mouzaki et al., 2019).

World Health Organization

The WHO published guidelines on the micronutrient intake in children with severe acute malnutrition. The guidelines recommend that the weight-for-height/weight-for-length status should be measured by clinicians to determine malnutrition. Micronutrient laboratory testing is not mentioned by the WHO.

The North American Expert Consensus Guidelines

A team of experts have published guidelines on breath tests including their use for a SIBO diagnosis. The authors have provided the following recommendations:

- "Current small bowel culture techniques are not satisfactory for the assessment of SIBO. [Quality of evidence: Low]
- If culture is considered for diagnosis of SIBO, based on the current evidence, we suggest the threshold of $>10^3$ c.f.u./ml for the definition of SIBO. [Quality of evidence: Low]
- We suggest breath testing in the diagnosis of small intestinal bacterial overgrowth. [Quality of evidence: Moderate]
- Until a true gold standard is established, we suggest breath testing in assessing the presence of antibiotic responsive microbial colonization of the gastrointestinal tract. [Quality of evidence: Moderate]
- We suggest to evaluate for excessive methane excretion on breath test in association with clinical constipation and slowing of gastrointestinal transit. [Quality of evidence: Moderate]
- We suggest that breath testing should not be used for assessment of orocecal transit time. [Quality of evidence: Moderate]
- We suggest breath testing for the diagnosis of carbohydrate maldigestion syndromes. [Quality of evidence: Moderate]

- We suggest breath testing in the assessment of conditions with bloating. [Quality of evidence: Low]
- We suggest that fructose and lactose breath test should be performed for at least 3 hours. [Quality of evidence: Moderate]
- We suggest that the presence of bacterial overgrowth should be ruled out before performing lactose or fructose breath testing [Quality of evidence: Moderate] (Rezaie et al., 2017)."

It may be worth noting that the above recommendation of LHBT testing for SIBO was publicly criticized by Usai-Satta et al. (2018) due to high false positive rates and a low sensitivity. The authors state that "in our opinion, LHBT should be neither recommended nor suggested to detect SIBO in the clinical practice. Despite a low sensitivity, Glucose BT [breath test] remains the most accurate BT for non-invasive diagnosis of SIBO (Usai-Satta et al., 2018)." In contrast, an article published in *Gastroenterology* by Baker et al. (2021) did a retroactive study, examining how these 2017 guidelines for glucose breath testing for SIBO compared to the older, modified Rome Consensus protocols. The authors found that the more recent North American Consensus protocol showed a higher percent of individuals with SIBO because of more prevalent positive methane excretion. Another article published by Pitcher et al. (2022) provide further support for the North American Consensus protocol for SIBO testing.

The Academy of Nutrition and Dietetics (AND)

The AND note that "serum proteins such as albumin and prealbumin are not included as defining characteristics of malnutrition because evidence analysis shows that serum levels of these proteins do not change in response to changes in nutrient intake. Hepatic proteins are not indicators of nutritional status, but are rather indicators of morbidity and mortality, and recovery from acute and chronic disease (AND, 2017)."

American College of Gastroenterology (ACG)

The ACG published an update on SIBO (Small Intestinal Bacterial Overgrowth). This guideline addresses diagnostic testing and treatment options for SIBO. Their recommendations include:

- "We suggest the use of breath testing (glucose hydrogen or lactulose hydrogen) for the diagnosis of SIBO in patients with IBS (conditional (weak) recommendation, very low level of evidence)."
- "We suggest using glucose hydrogen or lactulose hydrogen breath testing for the diagnosis of SIBO in symptomatic patients with suspected motility disorders (conditional (weak) recommendation, very low level of evidence)."
- "We suggest testing for SIBO using glucose hydrogen or lactulose hydrogen breath testing in symptomatic patients (abdominal pain, gas, bloating, and/or diarrhea) with previous luminal abdominal surgery (conditional (weak) recommendation, very low level of evidence)."
- "We suggest testing for methane using glucose or lactulose breath tests to diagnose the overgrowth of methane-producing organisms (IMO) in symptomatic patients with constipation (conditional (weak) recommendation, very low level of evidence)."

The ACG also notes that although "Small bowel aspirate and culture is often considered the gold standard for the diagnosis of SIBO," there have been some preliminary studies focusing on use of nucleic acid testing to diagnose SIBO. However, the ACG remarks that "Large-scale studies are currently underway to evaluate this further" (Pimentel et al., 2020).

VI. Applicable State and Federal Regulations

No specific U.S. Food and Drug Administration (FDA) approval or clearance of a test for idiopathic environmental intolerance was found. 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
82127	Amino acids; single, qualitative, each specimen
82136	Amino acids, 2 to 5 amino acids, quantitative, each specimen
82139	Amino acids, 6 or more amino acids, quantitative, each specimen
82379	Carnitine (total and free), quantitative, each specimen
82380	Carotene
82441	Chlorinated hydrocarbons, screen
82495	Chromium
82507	Citrate
82525	Copper
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
82656	Elastase, pancreatic (EL-1), fecal, qualitative or semi-quantitative
82705	Fat or lipids, feces; qualitative
82710	Fat or lipids, feces; quantitative
82715	Fat differential, feces, quantitative
82978	Glutathione
83150	Homovanillic acid (HVA)
83497	Hydroxyindolacetic acid, 5-(HIAA)
83918	Organic acids; total, quantitative, each specimen
83919	Organic acids; qualitative, each specimen
83921	Organic acid, single, quantitative
84134	Prealbumin

84255	Selenium
84585	Vanillylmandelic acid (VMA), urine
84600	Volatiles (eg, acetic anhydride, diethylether)
84630	Zinc
86001	Allergen specific IgG quantitative or semiquantitative, each allergen
86353	Lymphocyte transformation, mitogen (phytomitogen) or antigen induced blastogenesis
83015	Heavy metal (e.g., arsenic, barium, beryllium, bismuth, antimony, mercury); qualitative, any number of analytes
83018	Heavy metal (e.g., arsenic, barium, beryllium, bismuth, antimony, mercury); quantitative, each, not elsewhere specified
82108	Aluminum
82300	Cadmium
83735	Magnesium
83885	Nickel
83785	Manganese
82726	Very long chain fatty acids

89125	Fat stain, feces, urine, or respiratory secretions
82710	Fat or lipids, feces; quantitative
84590	Vitamin A
84446	Tocopherol alpha (Vitamin E)
83655	Lead
91065	Breath hydrogen or methane test (e.g., for detection of lactase deficiency, fructose intolerance, bacterial overgrowth, or oro-cecal gastrointestinal transit)

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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Diagnosis of Vaginitis including Multi-target PCR Testing

Policy #: AHS – M2057	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 1/19/22, 6/15/22 (See Section IX)

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

Vaginitis is defined as inflammation of the vagina with symptoms of discharge, itching, and discomfort often due to a disruption of the vaginal microflora. The most common infections are bacterial vaginosis, *Candida* vulvovaginitis, and trichomoniasis (Sobel, 1999). Other causes include vaginal atrophy in postmenopausal women, cervicitis, foreign body, irritants, and allergens (Sobel, 2020a).

Bacterial vaginosis (BV) is characterized by a shift in microbial species from the normally dominant hydrogen-peroxide producing *Lactobacillus* species to *Gardnerella vaginalis* and anaerobic commensals (Eschenbach et al., 1989; Hill, 1993; Lamont et al., 2011; Ling et al., 2010; Sobel, 2020b).

Vulvovaginal candidiasis (VVC) is characterized by *Candida* species. It is the second most common cause of vaginitis symptoms (after BV) and accounts for approximately one-third of vaginitis cases (Sobel, 2020c; Workowski & Bolan, 2015).

Trichomoniasis is caused by the flagellated protozoan *Trichomonas vaginalis*, which principally infects the squamous epithelium in the urogenital tract: vagina, urethra, and paraurethral glands (Kissinger, 2015; Sobel & Mitchell, 2020).

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.

Diagnosis of Vaginitis including Multi-target PCR Testing, continued

1. Testing of pH, testing for the presence of amines, saline wet mount, hydrogen peroxide (KOH) wet mount and microscopic examination of vaginal fluids **MEETS COVERAGE CRITERIA** in patients with symptoms of vaginitis.

2. Direct Probe DNA-based identification of *Gardnerella*, *Trichomonas*, and *Candida* **MEETS COVERAGE CRITERIA** in patients with symptoms of vaginitis.

3. Vaginal cultures for *Candida* species **MEET COVERAGE CRITERIA** for the diagnosis of vulvovaginal candidiasis in patients with clinical signs and symptoms of vaginitis and negative findings on wet-mount preparations and a normal pH test.

4. Measurement of sialidase activity in vaginal fluid **MEETS COVERAGE CRITERIA** for the diagnosis of bacterial vaginosis in women with symptoms of vaginitis.

5. Nucleic Acid Amplification Test (NAAT) or Polymerase Chain Reaction (PCR)-based identification of *Trichomonas vaginalis* **MEETS COVERAGE CRITERIA** in patients with symptoms of vaginitis.

6. Screening for *Trichomonas* **MEETS COVERAGE CRITERIA** for women with risk factors, including: new or multiple partners; history of sexually transmitted diseases (STDs); exchange of sex for payment; or injection drug use.

7. Polymerase Chain Reaction (PCR) based identification of *Candida* **MEETS COVERAGE CRITERIA** for any indication.

8. Nucleic Acid Amplification Test (NAAT), polymerase chain reaction (PCR) testing, and Multitarget PCR testing, when limited to known pathogenic species, **MEETS COVERAGE CRITERIA** for the diagnosis of bacterial vaginosis.

9. Screening for trichomoniasis and bacterial vaginosis **DOES NOT MEET COVERAGE CRITERIA** in asymptomatic patients, including asymptomatic pregnant patients at average or high risk for premature labor.

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. Rapid identification of *Trichomonas* by enzyme immunoassay **DOES NOT MEET COVERAGE CRITERIA** in patients with symptoms of vaginitis.

11. Using molecular-based panel testing, including, but not limited to testing such as SmartJane™, to test for microorganisms involved in vaginal flora imbalance and/or infertility **DOES NOT MEET COVERAGE CRITERIA**.

12. All other tests for vaginitis not addressed above **DO NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Vaginitis is characterized by several symptoms including odor, itching, abnormal vaginal discharge, burning and irritation; this inflammatory ailment is considered the most common gynecologic diagnosis in primary care as most women experience vaginitis at least once in their lives (Paladine & Desai, 2018). A diagnosis of vaginitis can be given based on a combination of symptoms, physical examination, and office or laboratory-based testing methods.

The squamous epithelium of the vagina in premenopausal women is rich in glycogen, a substrate for lactobacilli, which create an acidic vaginal environment (pH 4.0 to 4.5). This acidity helps maintain the normal vaginal flora and inhibits growth of pathogenic organisms. Disruption of the normal ecosystem by menstrual cycle, sexual activity, contraceptive, pregnancy, foreign bodies, estrogen level, sexually transmitted diseases, and use of hygienic products or antibiotics can lead to development of vaginitis. Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis are the three most common infections responsible for vaginitis. Other causes include: vaginal atrophy in postmenopausal women, cervicitis, foreign body, irritants and allergens (Sobel, 2020a).

Bacterial vaginosis is caused by an imbalance of naturally occurring vaginal bacteria, characterized by both a change in the most common type of bacteria present, along with an increase in the total number of bacteria present. Normal vaginal microbiota is dominated by the species *Lactobacilli*, which are known to produce hydrogen peroxide and lactic acid, which help to keep the acidic vaginal environment below pH 4.5 (Jones, 2019; Kairys & Garg, 2020). Though the origin of vaginal bacterial infections is still unclear, it is believed that most of such infections are the result of another bacteria, *Gardnerella vaginalis,* creating a biofilm which allows opportunistic bacteria to grow within the vagina, causing a decrease in the *Lactobacilli* and subsequent disruption of the pH of the system. An entire host of etiologic organisms have been identified as possible instigators and exacerbators, including *Atopobium vaginae*, *Megasphaera* phylotype 1 and 2, *Leptotrichia aminionii*, *Mobiluncus spp*, *Prevotella spp*, *Mycoplasma hominis*, *Bacteroides spp*, *Sneathia*, and BV-associated bacteria (BVAB)1, 2, and 3, though as aforementioned the causative mechanism and the interaction between these species are still uncertain (Jones, 2019).

Laboratory documentation of the etiology of vaginitis is important before initiating therapy, given the nonspecific nature and considerable overlap of the symptoms (Anderson, Klink, & Cohrssen, 2004; Ellis, Lerch, & Whitcomb, 2001; Landers, Wiesenfeld, Heine, Krohn, & Hillier, 2004). Diagnostic testing enables targeted treatment, increases therapeutic compliance, and increases the likelihood of partner notification (Sobel, 2020a; Workowski & Bolan, 2015).

Measurement of vaginal pH is the primary initial finding that drives the diagnostic. The pH of the normal vaginal secretions in premenopausal women with relatively high estrogen levels is 4.0 to 4.5. The pH of normal vaginal secretions in premenarchal and postmenopausal women in whom estrogen levels are low is ≥4.7. An elevated pH in a premenopausal woman suggests infections, such as BV (pH>4.5) or trichomoniasis (pH 5 to 6) and helps to exclude *Candida* vulvovaginitis (pH 4 to 4.5). Vaginal pH may also

be altered by lubricating gels, semen, douches, intravaginal medications and in pregnant women, leakage of amniotic fluid (Anderson et al., 2004; Sobel, 2020a).

Analytical Validity

Microscopic examination of normal vaginal discharge reveals a predominance of squamous epithelial cells, rare polymorphonuclear leukocytes (PMNs), and *Lactobacillus* species. The primary goal of the examination is to look for candidal buds or hyphae, motile trichomonads, epithelial cells studded with adherent coccobacilli (clue cells), and increased numbers of PMNs (Sobel, 2020a). The microscopic evaluation of BV is usually based on Amsel criteria (Amsel et al., 1983). Amsel criteria state that the presence of at least three out of the following four criteria are indicative of a BV diagnosis: increased homogeneous thin vaginal discharge, pH secretion > 4.5, amine odor when potassium hydroxide 10% solution is added to a vaginal secretion sample, and the presence of clue cells in wet preparations (Amsel et al., 1983). If clinical criteria are used to define infection, then reported sensitivity may range from 62 to 100 percent (Spiegel, 1991). Using Gram's stain as the standard for diagnosing BV, the sensitivity of Amsel criteria for diagnosis of BV is over 90 percent and specificity is 77 percent (Landers et al., 2004). The Nugent score is also available as a Gram staining scoring system to diagnose BV based on vaginal swab samples (Amegashie et al., 2017). Because BV represents complex changes in the vaginal flora, vaginal culture has **no** role in diagnosis. If microscopy is not available, commercial diagnostic testing methods (e.g., rapid antigen and nucleic acid amplification tests) are used for confirming the clinical suspicion of BV. Polymerase chain reaction (PCR)-based assays to quantify BV-associated bacteria (Cartwright et al., 2012; Menard, Fenollar, Henry, Bretelle, & Raoult, 2008) have good sensitivity and specificity compared with standard clinical tests (Dumonceaux et al., 2009; Menard et al., 2010). However, they are expensive and of limited utility (Sobel, 2020b).

Trichomoniasis can be diagnosed by the presence of motile trichomonads on wet mount, but it is identified in only 60 to 70 percent of culture-confirmed cases. Culture on Diamond's medium was considered the gold standard method for diagnosing a *T. vaginalis* infection (Workowski & Bolan, 2015); however, nucleic acid amplification tests (Baron et al., 2013) have become the accepted gold standard for the diagnosis of *T. vaginalis*. One study found the sensitivities for *T. vaginalis* using wet mount, culture, rapid antigen testing, and transcription-mediated amplification testing were 65, 96, 90, and 98 percent, respectively (Huppert et al., 2007). Coexistence of *T. vaginalis* and BV pathogens is common, with coinfection rates of 60 to 80 percent (Sobel & Mitchell, 2020; Sobel, Subramanian, Foxman, Fairfax, & Gygax, 2013).

Microscopy is negative in up to 50 percent of patients with culture-confirmed VVC (Sobel, 1985). Since there are no reliable point of care tests for *Candida* available in the United States (Abbott, 1995; Chatwani et al., 2007; Dan, Leshem, & Yeshaya, 2010; Hopwood, Evans, & Carney, 1985; Marot-Leblond et al., 2009; Matsui et al., 2009), culture must be obtained. PCR methods have high sensitivity and specificity and a shorter turn-around time than culture (Diba, Namaki, Ayatolahi, & Hanifian, 2012; Mahmoudi Rad, Zafarghandi, Amel Zabihi, Tavallaee, & Mirdamadi, 2012; Tabrizi, Pirotta, Rudland, & Garland, 2006; Weissenbacher et al., 2009), but they are costly and offer no proven benefit over culture in symptomatic women (Sobel, 2020c).

Lynch et al. (2019) collected vaginal swabs from 93 women in a cross-sectional study; results from microscopy were compared to two molecular approaches (a qPCR assay with a BV interpretive algorithm and a microbiome profiling test of the 16S rRNA gene produced by Illumina) (Lynch et al., 2019). Results show that "Microscopy plus BV Nugent score had 76% overall agreement with the qPCR plus BV interpretive algorithm method"; further, "Microscopic identification of *Candida* versus that by qPCR had 94% agreement (9 positive, 78 negative) (Lynch et al., 2019)." The qPCR assays gave additional information regarding the types of bacteria present, and the 16S microbiome analysis identified differentiating patterns between BV, aerobic vaginitis (AV), and *Lactobacillus* type infections.

Cartwright, Pherson, Harris, Clancey, and Nye (2018) have published data regarding the clinical validity of a PCR-based assay for the detection of BV. This multicenter study included 1579 patients and compared PCR results to samples realized by both the Nugent gram stain and a clinical evaluation using Amsel criteria. Next-generation sequencing was used to confirm differing results. After the resolution of discordant test results using next-generation sequencing, the BV-PCR assay reported a sensitivity of 98.7%, a specificity of 95.9%, a positive predictive value of 92.9% and a negative predictive value of 96.9% (Cartwright et al., 2018). These results show that this PCR-based assay can diagnose BV in symptomatic women efficiently.

Clinical Validity and Utility

As previously stated, microscopy, rather than bacterial culture, is the standard of care for diagnosing BV, and commercially available tests are available in the absence of microscopy but are not widely used. A study of 176 women using the Affirm VP III test (a DNA hybridization probe test that identifies high concentrations of *G. vaginalis*) reported comparable results to wet mount examination with no false positives and only three false negatives for *T. vaginalis,* and three false positives and four false negatives for *G. vaginalis* (Briselden & Hillier, 1994). This test "takes less than one hour to perform and is the best option when findings on physical examination suggest BV… but microscopy cannot be performed to look for clue cells (Sobel, 2020b)."

The OSOM BVBlue chromogenic diagnostic point-of-care test is a CLIA-waived test with a reported 10 minute read time. One study of 173 pregnant women reported a sensitivity and specificity of 94% and 96% respectively, as compared to Gram stain score (Sumeksri, Koprasert, & Panichkul, 2005). These results were comparable to the previously reported values of 91.7% sensitivity and 97.8% specificity in an earlier, smaller study of non-menstruating women (n=57) (Myziuk, Romanowski, & Johnson, 2003). A larger study (n=288 women) reported a sensitivity of 88% and specificity of 91% as compared to the Amstel criteria. The authors of this report concluded that women who "are not in settings where the conventional diagnostic methods are either practical or possible… would greatly benefit from access to rapid and reliable point-of-care tests to improve the diagnosis and management of BV (Bradshaw et al., 2005)."

The FDA approved the use of the BD MAX Vaginal Panel as "an automated qualitative *in vitro* diagnostic test for the direct detection of DNA targets from bacteria associated with BV (qualitative results reported based on detection and quantitation of targeted organism markers), *Candida* species associated with vulvovaginal candidiasis, and *Trichomonas vaginalis* from vaginal swabs in patients who

are symptomatic for vaginitis/vaginosis. The test utilizes real-time PCR for the amplification of specific DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA (FDA, 2016)." A 2017 cross-sectional, multi-site study into the clinical validation of this system (n=1740 symptomatic women) reported a sensitivity and specificity of 90.9% and 94.1%, respectively for the *Candida* group and 90.5% sensitivity and 85.8% specificity for BV. For *C. glabrata* specifically, the assay had only 75.9% sensitivity but 99.7% specificity. For trichomoniasis, the sensitivity and specificity were 93.1% and 99.3%, respectively (Gaydos et al., 2017). These researchers also compared the results of this test to clinician assessment. Again, to qualify for the study, the women must have at least one symptom of BV. Using Amsel's criteria, the investigational test sensitivity was 92.7% as compared to the 75.6% sensitivity of the clinician assessment. The authors conclude, "The investigational test showed significantly higher sensitivity for detecting vaginitis, involving more than one cause, than did clinician diagnosis. Taken together, these results suggest that a molecular investigational test can facilitate accurate detection of vaginitis (Schwebke et al., 2018)." It should be noted, however, that these studies only included symptomatic women, and, therefore, the possible clinical nonspecificity (i.e. instances where an asymptomatic woman would test positive) is not addressed. Sherrard (2019) compared BV, candidiasis, and trichomoniasis diagnostic results from the BD MAX Vaginal Panel to a current test used in a UK specialist sexual health service center. The authors reported that the BD MAX Vaginal Panel had a sensitivity of 86.4% and specificity of 86.0% for *Candida* species, and a sensitivity of 94.4% and specificity of 79% for BV; the specificity for BV was lower in this study than what has been previously reported (Sherrard, 2019).

SureSwab®(Quest Diagnostics, Inc.) is a multi-target PCR test using RT-PCR to screen for a number of microorganisms involved in vaginal flora imbalances, including *B. vaginalis, T. vaginalis, C. albicans, C. glabrata, C. tropicalis,* and *C. parapsilosis*, from a vaginal swab. The swab can be collected either by a physician or the patient (Quest, 2019a). Similarly, Quest Diagnostics also offers the SureSwab® Vaginosis/Vaginitis Plus test, which tests for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in addition to the microorganisms of the SureSwab® test (Quest, 2019b). The test is based on the unique set of primers synthesized by a CDC research team to identify *Candida* that purports to diagnose vulvovaginal candidiasis while ruling out other genital infections (CDC, 2016). The CDC research group, led by Dr. C.J. Morrison, developed the DNA probes to identify medically important *Candida* species by the internal transcribed spacer 2 region of ribosomal DNA. The specific hybridization was measured by a sample-to-background ratio of 58.7, 53.2, 46.9, 59.9, and 54.7 for *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei*, respectively. The negative control sample-to-background ratio was 0.9 (Das, Brown, Kellar, Holloway, & Morrison, 2006).

The OSOM *Trichomonas vaginalis* (TV) Rapid Test by Sekisui Diagnostics is "an antigen-detection test using immunochromatographic capillary flow dipstick technology that can be performed at the point of care (CDC, 2015b)." The diagnostic accuracy of the OSOM TV Rapid assay was tested against the common laboratory-based Anyplex II STI-7 Detection in a South African cross-sectional study; all irregular results were further tested with the Fast Track Diagnostics (FTD) STD9 assay (Garrett et al., 2019). Vaginal swabs from 247 women were tested for this study. "The sensitivity and specificity of

OSOM TV were 75.0% (45.0-100) and 100% (100-100)", respectively, showing a very high specificity and lower sensitivity (Garrett et al., 2019).

The AMPLISwab™ by MedLabs is a comprehensive test created to assess the different organisms responsible for a variety of female genital tract infections, including causative pathogens for cervicitis, nongonococcal urethritis, pelvic inflammatory disease and infertility, sexually transmitted infections, and vaginitis (e.g., bacterial vaginosis, candidiasis and trichomoniasis). The test requires one swab to test for 23 total organisms, broken down into four categories (7 yeast, 12 bacteria and 1 reference bacteria, 1 parasite, and 2 types of herpes viruses), employing testing methodologies such as automated DNA/RNA extraction, transcription mediated amplification (TMA), and real-time polymerase chain reaction (RT-PCR) for the quantification of select organisms implicated in bacterial vaginosis (MedLabs, 2015).

The multiplex PCR assay SmartJane™ measures a specimen's vaginal flora (such as *Lactobacillus iners* or *Treponema pallidum*). The test proposes that the results can provide a health snapshot of the environment tested based on the levels of microorganisms detected. The procedure for the test requires the user to self-sample by collecting a vaginal swab and sending the sample back to Ubiome where it is analyzed. The labs use Precision Sequencing technology to extract DNA from the microorganisms in the sample and Illumina Next-Generation to sequence the targeted genes. Then, phylogenetic algorithms are used to analyze and organize the DNA from those microorganisms. Finally, a clinical report detailing the levels of the targeted microorganisms is sent to the user and medical provider (Ubiome, 2018). The report contains measurements of its targeted microorganisms, informing the patient whether those measurements are within the normal reference ranges for certain conditions, and whether certain high danger pathogens are present. The manufacturers state that on average SmartJane™ has a sensitivity and specificity for the species of microorganism of 99.4% and 100.0%, respectively. SmartJane™ tests for 19 different HPV strains and common pathological agents involved in sexually transmitted infections in addition to more than 20 different microorganisms involved in BV, including *G. vaginalis* (Ubiome, 2017).

Even though studies have shown that PCR methods have a higher specificity and sensitivity than culture and shorter turn-around time in identifying *Candida* (Diba et al., 2012; Mahmoudi Rad et al., 2012; Tabrizi et al., 2006; Weissenbacher et al., 2009), their use may be adding to clinical nonspecificity. Tabrizi et al. (2006) reported that PCR "detected four additional *Candida albicans*, three *Candida parapsilosis* and one *Candida tropicalis* when compared with culture. All but one case additionally detected by PCR were found in patients with no VVC symptoms (Tabrizi et al., 2006)." These data support the earlier findings by Giraldo et al. (2000) where, unlike culture testing, "*Candida* was identified by PCR in a similar proportion of patients with previous recurrent vulvovaginal candidiasis (30%) and in controls (28.8%)."

IV. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

Diagnosis of Vaginitis including Multi-target PCR Testing, continued

The CDC published recommendations for the evaluation of diseases characterized by vaginal discharge in the 2015 Sexually Transmitted Diseases Treatment Guidelines (CDC, 2015a). These guidelines state, "Various diagnostic methods are available to identify the etiology of an abnormal vaginal discharge…In the clinician's office, the cause of vaginal symptoms might be determined by pH, a potassium hydroxide (KOH) test, and microscopic examination of fresh samples of the discharge" and "In settings where pH paper, KOH, and microscopy are not available, alternative commercially available point-of-care tests or clinical laboratory testing can be used to diagnose vaginitis (CDC, 2015a)."

For the evaluation of BV, the CDC recommends that "BV can be diagnosed by the use of clinical criteria (i.e., Amsel's Diagnostic Criteria) (Amsel et al., 1983) or Gram stain"; further, "Other tests, including Affirm VP III (Becton Dickinson, Sparks, MD), a DNA hybridization probe test for high concentrations of *G. vaginalis*, and the OSOM BV Blue test (Sekisui Diagnostics, Framingham, MA), which detects vaginal fluid sialidase activity, have acceptable performance characteristics compared with Gram stain. Although a prolineaminopeptidase card test is available for the detection of elevated pH and trimethylamine, it has low sensitivity and specificity and therefore is not recommended. PCR has been used in research settings for the detection of a variety of organisms associated with BV, but evaluation of its clinical utility is still underway. Detection of specific organisms might be predictive of BV by PCR (Cartwright et al., 2012; Fredricks, Fiedler, Thomas, Oakley, & Marrazzo, 2007). Additional validation is needed before these tests can be recommended to diagnose BV. Culture of *G. vaginalis* is not recommended as a diagnostic tool because it is not specific. Cervical Pap tests have no clinical utility for the diagnosis of BV because of their low sensitivity and specificity (CDC, 2015a)." The guidelines also state that "evidence is insufficient to recommend routine screening for BV in asymptomatic pregnant women at high or low risk for preterm delivery for the prevention of preterm birth (CDC, 2015a)", which is in compliance with the 2008 USPSTF recommendations (USPSTF, 2008).

For the evaluation of vulvovaginal candidiasis, the CDC recommends: "Examination of a wet mount with KOH preparation should be performed for all women with symptoms or signs of VVC, and women with a positive result should be treated. For those with negative wet mounts but existing signs or symptoms, vaginal cultures for *Candida* should be considered…PCR testing for yeast is not FDA-cleared; culture for yeast remains the gold standard for diagnosis (CDC, 2015c)." DNA hybridization probe tests are not addressed.

For the evaluation of Trichomoniasis, the CDC recommends: "Diagnostic testing for *T. vaginalis* should be performed in women seeking care for vaginal discharge… The use of highly sensitive and specific tests is recommended for detecting *T. vaginalis.* Among women, NAAT is highly sensitive, often detecting three to five times more *T. vaginalis* infections than wet-mount microscopy, a method with poor sensitivity (51%–65%) (CDC, 2015b; Hollman, Coupey, Fox, & Herold, 2010; Roth et al., 2011)." Regarding point of care testing, it is stated that "Other FDA-cleared tests to detect *T. vaginalis* in vaginal secretions include the OSOM *Trichomonas* Rapid Test (Sekisui Diagnostics, Framingham, MA), an antigen-detection test using immunochromatographic capillary flow dipstick technology that can be performed at the point of care, and the Affirm VP III (Becton Dickinson, Sparks, MD), a DNA hybridization probe test that evaluates for *T. vaginalis*, *G. vaginalis*, and *Candida albicans*. The results of the OSOM *Trichomonas* Rapid Test are available in approximately 10 minutes, with sensitivity 82%–95% and specificity 97%–100% (Campbell, Woods, Lloyd, Elsayed, & Church, 2008; Huppert et al., 2007). Self-testing might

become an option, as a study of 209 young women aged 14‑22 years found that >99% could correctly perform and interpret her own self-test using the OSOM assay, with a high correlation with clinician interpretation (96% agreement, κ = 0.87) (Huppert et al., 2010). The results of the Affirm VP III are available within 45 minutes. Sensitivity and specificity are 63% and 99.9%, respectively, compared with culture and TMA; sensitivity might be higher among women who are symptomatic (Andrea & Chapin, 2011; Brown, Fuller, Jasper, Davis, & Wright, 2004; CDC, 2015b)."

American Academy of Family Physicians (AAFP)

The AAFP published an article (Hainer & Gibson, 2011) on the diagnosis of vaginitis which states that: "Physicians traditionally diagnose vaginitis using the combination of symptoms, physical examination, pH of vaginal fluid, microscopy, and the whiff test. When combined, these tests have a sensitivity and specificity of 81 and 70 percent, respectively, for BV; 84 and 85 percent for vulvovaginal candidiasis; and 85 and 100 percent for trichomoniasis when compared with the DNA probe standard…A cost-effectiveness analysis of diagnostic strategies for vaginitis undiagnosed by pelvic examination, wet-mount preparation, and related office tests showed that the least expensive strategy was to perform yeast culture, gonorrhea and chlamydia probes at the initial visit, and Gram stain and *Trichomonas* culture only when the vaginal pH exceeded 4.9. Other strategies cost more and increased duration of symptoms by up to 1.3 days (Hainer & Gibson, 2011)."

In 2018, the AAFP has published the following guidelines:

- "Symptoms alone cannot differentiate between the causes of vaginitis. Office-based or laboratory testing should be used with the history and physical examination findings to make the diagnosis. (C evidence rating)
- Do not obtain culture for the diagnosis of bacterial vaginosis because it represents a polymicrobial infection. (C evidence rating)
- Nucleic acid amplification testing is recommended for the diagnosis of trichomoniasis in symptomatic or high-risk women. (C evidence rating) (Paladine & Desai, 2018)."

U.S. Preventive Services Task Force Recommendations (USPSTF)

In 2020, the USPSTF published recommendations discouraging the use of screening for BV in pregnancy: "The USPSTF recommends against screening for bacterial vaginosis in pregnant persons not at increased risk for preterm delivery". On a similar note, the USPSTF maintains its 2008 recommendation stating "that the current evidence is insufficient to assess the balance of benefits and harms of screening for bacterial vaginosis in pregnant persons at increased risk for preterm delivery" (Owens et al., 2020).

American College of Obstetrics and Gynecology (ACOG)

ACOG published recommendations (ACOG, 2006) for the evaluation of vaginitis in 2006, and reaffirmed in 2017 (ACOG, 2017, 2018), which state: "Evaluation of women with vaginitis should include a focused history about the entire spectrum of vaginal symptoms, including change in discharge, vaginal malodor, itching, irritation, burning, swelling, dyspareunia, and dysuria." Further, "During speculum examination, samples should be obtained for vaginal pH, amine ("whiff") test, and saline (wet mount) and 10% potassium hydroxide (KOH) microscopy. The pH and amine testing can be performed either through

direct measurement or by colorimetric testing." With a Level B recommendation, ACOG states, "Microscopy is the first line for diagnosing vulvovaginal candidiasis and trichomoniasis. In selected patients, culture for yeast and *T. vaginalis* should be obtained in addition to standard office-based testing." Additionally, "A vaginal Gram stain for Nugent scoring of the bacterial flora may help to identify patients with BV. Other currently available ancillary tests for diagnosing vaginal infections include rapid tests for enzyme activity from BV-associated organisms, *Trichomonas vaginalis* antigen, and point-of care testing for DNA of *G. vaginalis*, *T. vaginalis*, and *Candida* species; however, the role of these tests in the proper management of patients with vaginitis is unclear. Depending on risk factors, DNA amplification tests can be obtained for *Neisseria gonorrheae* and *Chlamydia trachomatis* (ACOG, 2006)."

The ACOG published in 2020 Practice Bulletin Number 215 on vaginitis in nonpregnant patients. In these guidelines, the ACOG made these recommendations for diagnostic testing based on good and consistent scientific evidence (Level B):

- "The use of Amsel clinical criteria or Gram stain with Nugent scoring is recommended for the diagnosis of bacterial vaginosis."
- "Nucleic acid amplification testing is recommended for the diagnosis of trichomoniasis."
- "In a symptomatic patient, diagnosis of vulvovaginal candidiasis requires one of the following two findings: 1) visualization of spores, pseudohyphae, or hyphae on wet-mount microscopy or 2) vaginal fungal culture or commercial diagnostic test results positive for *Candida* species."

The ACOG also published recommendations based on limited or inconsistent scientific evidence (Level B), along with a series of recommendations based on consensus and expert opinion (Level C). Those relating to diagnostic testing are reported below:

- "Patients should be retested within 3 months after treatment for *T vaginalis* because of the high rates of infection recurrence." (Level B)
- "Pap tests are not reliable for the diagnosis of vaginitis. Diagnostic confirmation is recommended for incidental findings of vulvovaginal candidiasis, bacterial vaginosis, or trichomoniasis on a Pap test." (Level B)
- "A complete medical history, physical examination of the vulva and vagina, and clinical testing of vaginal discharge (ie. pH testing, a potassium hydroxide [KOH] "whiff test", and microscopy) are recommended for the initial evaluation of patients with vaginitis symptoms." (Level C)

Infectious Diseases Society of America (IDSA) Clinical Practice Guidelines

IDSA has published an updated clinical guideline (Pappas et al., 2016) for the management of candidiasis in which recommendations include diagnosing vulvovaginal candidiasis before proceeding with empiric antifungal therapy. The usual diagnosis is clinical based on signs and symptoms of vaginitis such as pruritus, irritation, vaginal soreness, vulvar edema, erythema and many others. Clinical signs and symptoms are nonspecific and could be attributed to causes other than vulvovaginal candidiasis. Therefore, authors recommend confirming clinical diagnosis by a wet -mount preparation with saline and 10% KOH to demonstrate the presence of yeast and a normal pH. In cases where signs and symptoms are suggestive of vulvovaginal candidiasis, but microscopic findings and pH are negative, culture testing confirms the diagnosis according to published guidelines. The IDSA also discusses the possible use of PCR in diagnosing invasive candidiasis, even though the guidelines later state that "Cultures of blood or other samples collected under sterile conditions have long been considered

diagnostic gold standards for invasive candidiasis…The role of PCR in testing samples other than blood is not established (Pappas et al., 2016)."

In the 2018 IDSA *A guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases*, the IDSA states, "For vaginosis (altered vaginal flora) a Gram stain and recently available microbiome-based assays are more specific than culture and probe testing for *Gardnerella vaginalis* alone… A number of point-of-care tests can be performed from a vaginal discharge specimen while the patient is in the healthcare setting. Although point-of-care tests are popular, the sensitivity and specificity for making a specific diagnosis vary widely and these assays, while rapid, are often diagnostically poor (Miller et al., 2018)." The IDSA notes that the FDA has approved the use of the Max Vaginal Panel by Becton Dickinson in symptomatic females. "Preliminary data show greater specificity of this approach compared to methods that identify only *G. vaginalis*, as well as consistency in both reproducible as well as standardized results (Miller et al., 2018)."

Society of Obstetricians and Gynecologists of Canada (SOGC)

The SOGC published guidelines for the screening and management of BV in pregnancy. These guidelines state that the following:

- "In symptomatic pregnant women, testing for and treatment of bacterial vaginosis is recommended for symptom resolution. Diagnostic criteria are the same for pregnant and non-pregnant women (I-A).
- Asymptomatic women and women without identified risk factors for preterm birth should not undergo routine screening for or treatment of bacterial vaginosis (I-B).
- Women at increased risk for preterm birth may benefit from routine screening for and treatment of bacterial vaginosis (I-B).
- Testing should be repeated one month after treatment to ensure that cure was achieved (III-L) (Yudin & Money, 2017)."

The SOGC also published guidelines regarding the screening and management of trichomoniasis, VVC, and BV. These guidelines state that "Bacterial vaginosis should be diagnosed using either clinical (Amsel's) or laboratory (Gram stain with objective scoring system) criteria (II-2A) (van Schalkwyk & Yudin, 2015)."

V. State and Federal Regulations (as applicable)

A search of the term "vaginitis" on the FDA Device database on 01/29/2021 yielded 145 records. 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

Diagnosis of Vaginitis including Multi-target PCR Testing, continued

Code Number	Code Description
81513	Infectious disease, bacterial vaginosis, quantitative real-time amplification of RNA markers for Atopobium vaginae, Gardnerella vaginalis, and Lactobacillus species, utilizing vaginal-fluid specimens, algorithm reported as a positive or negative result for bacterial vaginosis Proprietary test: Aptima® BV Assay Lab/Manufacturer: Hologic, Inc
81514	Infectious disease, bacterial vaginosis and vaginitis, quantitative real-time amplification of DNA markers for Gardnerella vaginalis, Atopobium vaginae, Megasphaera type 1, Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), and Lactobacillus species (L. crispatus and L. jensenii), utilizing vaginal-fluid specimens, algorithm reported as a positive or negative for high likelihood of bacterial vaginosis, includes separate detection of Trichomonas vaginalis and/or Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata, Candida krusei, when reported Proprietary test: BD MAX™ Vaginal Panel Lab/Manufacturer: Becton Dickson and Company
82120	Amines, vaginal fluid, qualitative
83986	Ph; body fluid, not otherwise specified
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, 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
87210	Smear, primary source with interpretation; wet mount for infectious agents (e.g., saline, India ink, KOH preps)
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 technique
87510	Infectious agent detection by nucleic acid (dna or rna); gardnerella vaginalis, direct probe technique
87511	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, amplified probe technique
87512	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, quantification
87660	Infectious agent detection by nucleic acid (dna or rna); trichomonas vaginalis, direct probe technique
87661	Infectious agent detection by nucleic acid (dna or rna); trichomonas vaginalis, amplified probe technique

87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, 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
87808	Infectious agent antigen detection by immunoassay with direct optical (i.e., visual) observation; Trichomonas vaginalis
87905	Infectious agent enzymatic activity other than virus (e.g., sialidase activity in vaginal fluid)
0330U	Infectious agent detection by nucleic acid (DNA or RNA), vaginal pathogen panel, identification of 27 organisms, amplified probe technique, vaginal swab Proprietary test: Bridge Women's Health Infectious Disease Detection Test Lab/Manufacturer: Bridge Diagnostics/ThermoFisher and Hologic Test Kit on Panther Instrument
Q0111	Wet mounts, including preparations of vaginal, cervical or skin specimens

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
1/11/22	Added coverage criteria #8 and #12; added CPT code 87801 (Infectious agent detection by nucleic acid [DNA or RNA], multiple organisms; amplified probe[s] technique)
6/15/22	Changed coverage criteria #7 from **DOES NOT MEET COVERAGE CRITERIA** to **MEETS COVERAGE CRITERIA**.

Diagnostic Testing of Common Sexually Transmitted Infections

Policy #: AHS – G2157	**Prior Policy Name & Number (as applicable):** Portions of this policy replaces portions of M2097- Identification of Microorganisms using Nucleic Acid Probes
Implementation Date: 9/15/21	**Date of Last Revision:** 11/12/21, 2/13/23, 10/3/23, 1/8/24 (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

Sexually transmitted infections (STIs), often referred to as sexually transmitted diseases or STDs, include a variety of pathogenic bacteria, virus, and other microorganisms that are spread through sexual contact and can cause a multitude of complications if left untreated. Chlamydia and gonorrhea, caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, respectively, have high rates of occurrence in the United States and can cause pelvic inflammatory disease (PID), infertility, and pregnancy complications. The causative agent of syphilis is *Treponema pallidum*; if left untreated, syphilis can lead to serious cardiac and neurological conditions (Ghanem & Tuddenham, 2023). Human papillomavirus (HPV) is a double-stranded DNA virus that can be sexually transmitted and is associated with cervical cancer, vulvar/vaginal cancer, anal cancer, oropharyngeal cancer, penile cancer, and both genital and non-genital warts. "Globally, anogenital HPV is the most common sexually transmitted infection" with an estimated 80% of sexually active adults exposed to it at least once in their lifetime (Palefsky, 2022). Herpes simplex virus (HSV) is a common STI where many individuals are asymptomatic. HSV infection has been linked to an increased risk of other infections, including HIV, and in rare cases, can also result in HSV meningitis or proctitis (Albrecht, 2022). In general, risk factors for STIs can include both behavioral elements, such as multiple sex partners, working in a sex trade, and inconsistent use of condoms when in non-monogamous relationships as well as demographic risks, including men who have sex with men (MSM), prior STI diagnosis, admission to correctional facilities, and lower socioeconomic status (Ghanem & Tuddenham, 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) Antibody testing for syphilis infection **MEETS COVERAGE CRITERIA** in the following situations:
   a) For any asymptomatic person in a high-risk category (see Notes 1 & 2), once a year assessment using either a "standard" or "reverse" algorithm that includes initial and confirmatory tests for any initial positive test, such as:
      i) Treponemal Ig test; and
      ii) Nontreponemal Ig test.
   b) For diagnosis of any person presenting with signs and/or symptoms of a syphilis infection (see Note 3).
   c) Once every three months for HIV-positive men or MSM.
   d) Treponemal Ig testing and nontreponemal testing (once prior to transplant) as a part of a pre-transplant assessment in both donors and recipients of an allogeneic hematopoietic stem cell transplantation (allo-HCT).
   e) When a nontreponemal test is used as a test of cure (TOC) for a positive syphilis infection.
2) For asymptomatic individuals NOT belonging to a high-risk category (see Notes 1 & 2), antibody screening for syphilis **MEETS COVERAGE CRITERIA** only in the following situations:
   a) As part of newborn screening.
   b) As part of follow-up in a victim of sexual assault.
   c) For sexually active individuals less than 18 years of age (annually).
3) Polymerase chain reaction (PCR) testing and nucleic acid amplification testing (NAAT) for syphilis **DO NOT MEET COVERAGE CRITERIA**.
4) NAAT for chlamydia **MEETS COVERAGE CRITERIA** in the following situations:
   a) Once a year assessment for any asymptomatic person in a high-risk category (see Notes 1 & 4).

b) For diagnosis of any person presenting with signs and/or symptoms of a chlamydial infection (see Note 5).

c) For the diagnosis of any person with suspected lymphogranuloma venereum (LGV).

d) At least three months after initial chlamydial diagnosis as a TOC.

5) For asymptomatic individuals NOT belonging to a high-risk category (see Notes 1 & 4), screening for chlamydia **MEETS COVERAGE CRITERIA** only in the following situations:

a) As part of newborn screening.

b) As part of follow-up in a victim of sexual assault.

c) For sexually active individuals less than 18 years of age (annually).

6) Serology testing for chlamydia or LGV **DOES NOT MEET COVERAGE CRITERIA**.

7) NAAT for gonorrhea **MEETS COVERAGE CRITERIA** in the following situations:

a) Once a year assessment for any asymptomatic person in a high-risk category (see Notes 1 & 4).

b) For diagnosis of any person presenting with signs and/or symptoms of a gonorrheal infection (see Note 6).

c) As a TOC for treatment.

8) For an individual that does not respond to initial treatment, culture testing for *N. gonorrhoeae* to determine antimicrobial susceptibility **MEETS COVERAGE CRITERIA**.

9) For asymptomatic individuals NOT belonging to a high-risk category (see Notes 1 & 4), screening for gonorrhea **MEETS COVERAGE CRITERIA** only in the following situations:

a) As part of newborn screening.

b) As part of follow-up in a victim of sexual assault.

c) For sexually active individuals less than 18 years of age (annually).

10) NAATs or PCR-based testing for *T. vaginalis* **MEETS COVERAGE CRITERIA** in the following situations:

a) Symptomatic individuals (see Note 7).

b) Asymptomatic individuals belonging to a high-risk group:

i) Concurrent STI or history of STIs.

ii) Individuals in high prevalence settings, such as STI clinics.

iii) Individuals who exchange sex for payment.

11) Rapid identification of Trichomonas by enzyme immunoassay **DOES NOT MEET COVERAGE CRITERIA**.

Diagnostic Testing of Common Sexually Transmitted Infections, continued

12) For symptomatic individuals (see Note 8), testing for *Mycoplasma genitalium* using NAAT **MEETS COVERAGE CRITERIA.**

13) For asymptomatic individuals (see Note 8), screening for *M. genitalium* using NAAT **DOES NOT MEET COVERAGE CRITERIA.**

14) When an individual meets any of the conditions described above multitarget PCR testing (targets limited to *C. trachomatis*, *N. gonorrhoeae, T. vaginalis, and M. genitalium*) **MEETS COVERAGE CRITERIA.**

15) For individuals with active genital ulcers or mucocutaneous lesions, nucleic acid amplification testing (NAAT) for herpes simplex virus-1 (HSV-1) or herpes simplex virus-2 (HSV-2) **MEETS COVERAGE CRITERIA**.

16) Immunoassay testing for HSV-1 and and/or herpes simplex (non-specific type test) **DOES NOT MEET COVERAGE CRITERIA**.

17) Type-specific serologic testing for HSV-2 using a glycoprotein G2 (gG2) test **MEETS COVERAGE CRITERIA** in the following situations:

    a) Recurrent or atypical genital symptoms or lesions in individuals with a negative herpes simplex virus PCR or culture result.

    b) For the clinical diagnosis of genital herpes in individuals with a negative PCR or culture result or without laboratory confirmation.

    c) When an individual's partner has genital herpes.

18) In asymptomatic individuals, screening for HSV-1 or HSV-2 **DOES NOT MEET COVERAGE CRITERIA**.

19) In the diagnosis and/or assessment of cancer or cancer therapy (immunohistochemistry testing for p16 or NAAT testing for high-risk human papillomavirus [HR-HPV]), testing for HPV **MEETS COVERAGE CRITERIA**.

20) Testing for HPV **DOES NOT MEET COVERAGE CRITERIA** in the following situations:

    a) To screen for oncogenic high-risk types, such as HPV-16 and HPV-18, as part of a general sexually transmitted disease (STD) or sexually transmitted infection (STI) screening process **or** panel for asymptomatic individuals.

    b) As part of the diagnosis of anogenital warts.

    c) To screen for low-risk types of HPV.

    d) In the general population, either as a part of a panel of tests **or** as an individual NAAT to determine HPV status.

21) Prior to beginning a preexposure prophylaxis (PrEP) regimen, the following screens/test **MEET COVERAGE CRITERIA:**

a) Serum creatinine and estimated creatinine clearance to determine baseline renal function.

b) Antibody screening to confirm a baseline negative antibody result for HIV.

c) Hepatitis B (HBV) and/or Hepatitis C screening to identify positive individuals.

d) Pregnancy testing.

22) While an individual is undergoing a preexposure prophylaxis (PrEP) regimen for HIV prevention, the following screens/tests **MEET COVERAGE CRITERIA**:

a) A blood test once every three months to confirm a negative antibody result for HIV.

b) Serum creatinine and estimated creatinine clearance three months after beginning PrEP and up to one time every six months thereafter to assess renal function.

c) NAAT screening, based on anatomic site of exposure, for gonorrhea and chlamydia:

i) Once every three months for MSM and for individuals with child-bearing potential.

ii) Nine months after PrEP is initiated and once every six months thereafter for sexually active individuals

d) Blood test to screen for syphilis once every three months in MSM and individuals with child-bearing potential.

i) Once every three months for MSM and for individuals with child-bearing potential.

a. As part of follow-up of victim of sexual assault.

ii) Nine months after PrEP is initiated and once every six months thereafter for sexually active individuals

e) Pregnancy testing once every three months.

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.

23) Using nucleic acid testing to quantify the following microorganisms **DOES NOT MEET COVERAGE CRITERIA**:

a) *Chlamydia trachomatis*

b) *Neisseria gonorrhoeae*

c) Herpes Simplex Virus-1

d) Herpes Simplex Virus-2

e) Human Papillomavirus

f) *Treponema pallidum*

Diagnostic Testing of Common Sexually Transmitted Infections, continued

NOTES:

Note 1: For sexually active children and adolescents under the age of 18, risk factors for chlamydia, gonorrhea, and/or syphilis infection as defined by the CDC include: initiating sex early in adolescence; living in detention facilities; receiving services at STD clinics; being involved in commercial sex exploitation or exchanging sex for drugs, money, food, or housing; having multiple sex partners, having sequential sex partnerships of limited duration or concurrent partnerships; failing to use barrier protection consistently and correctly; having lower socioeconomic status, and facing numerous obstacles to accessing healthcare. At-risk individuals also include: males who have sex with males (YMSM); transgender youths; youths with disabilities, substance abuse, or mental health disorders (CDC, 2022b).

NOTE 2: High-risk for Syphilis (Cantor et al., 2016; CDC, 2023d):

- Sexually active men who have sex with men (MSM)
- Sexually active HIV-positive status
- Having a sexual partner recently diagnosed with an STI
- Exchanging sex for money or drugs
- Individuals in adult correctional facilities
- During pregnancy when the following risk factors are present:
  - Sexually active HIV-positive status
  - Sexually active with multiple partners
  - Sexually active in conjunction with drug use or transactional sex
  - Late entry to prenatal care (i.e., first visit during the second trimester or later) or no prenatal care
  - Methamphetamine or heroin use
  - Incarceration of the woman or her partner
  - Unstable housing or homelessness

NOTE 3: Signs and Symptoms of a Syphilis Infection (CDC, 2018, 2023d)

- Chancre
- Skin rash and/or mucous membrane lesions in mouth, vagina, anus, hands, and feet
- Condyloma lata
- Secondary symptomology can include fever, fatigue, sore throat, swollen lymph nodes, weight loss, muscle aches, headache, and hair loss

- Signs and symptoms of neurosyphilis can include severe headache, trouble with muscle movements, muscle weakness or paralysis (not being able to move certain parts of the body), numbness, and changes in mental status (trouble focusing, confusion, personality change) and/or dementia (problems with memory, thinking, and/or making decisions).
- Signs and symptoms of ocular syphilis can include eye pain or redness, floating spots in the field of vision ("floaters"), sensitivity to light, and changes in vision (blurry vision or even blindness).
- Signs and symptoms of otosyphilis may include hearing loss, ringing, buzzing, roaring, or hissing in the ears ("tinnitus"), balance difficulties, and dizziness or vertigo.

- Signs and symptoms of late/tertiary syphilis include inflammatory lesions of the cardiovascular system (e.g., aortitis, coronary vessel disease), skin (e.g., gummatous lesions), and bone (e.g., osteitis).

NOTE 4: High-risk for Chlamydia and/or Gonorrhea (CDC, 2021d, 2023a, 2023b; LeFevre, 2014):

- Sexually active men who have sex with men (MSM)
- Sexually active HIV-positive status
- Sexually active women under the age of 25
- Women age 25 or over who have multiple sexual partners
- Having a sexual partner recently diagnosed with an STI
- Previous or concurrent STI
- Exchanging sex for money or drugs

NOTE 5: Signs and Symptoms of a Chlamydia Infection (CDC, 2021d, 2023a):

- Genital symptoms, including "discharge, burning during urination, unusual sores, or rash"
- Pelvic Inflammatory Disease, including "symptoms of abdominal and/or pelvic pain, along with signs of cervical motion tenderness, and uterine or adnexal tenderness on examination"
- Urethritis
- Pyuria
- Dysuria
- Increase in frequency in urination
- Epididymitis (with or without symptomatic urethritis) in men
- Proctitis
- Sexually acquired chlamydial conjunctivitis

NOTE 6: Signs and Symptoms of Gonorrhea (CDC, 2023b):

- Dysuria
- Urethral infection
- Urethral or vaginal discharge
- Epididymitis (Testicular or scrotal pain)
- Rectal infection symptoms include anal itching, discharge, rectal bleeding, and painful bowel movements

NOTE 7: Signs and Symptoms of Trichomoniasis (CDC, 2021h, 2022e):

- Vaginal or penile discharge
- Itching, burning sensation, or soreness of the genitalia
- Discomfort or burning sensation during/after urination and/or ejaculation
- Urethritis
- Epididymitis

- Prostatitis

Note 8: Signs and Symptoms of *M. genitalium* Infection (CDC, 2022c):

- When present, typical symptoms of *Mgen*-urethritis in men include dysuria, urethral pruritus, and purulent or mucopurulent urethral discharge
- When present, typical symptoms of *Mgen* cervicitis in women include vaginal discharge, vaginal itching, dysuria, and pelvic discomfort
- When present, typical symptoms of PID due to *Mgen* include mild to severe pelvic pain, abdominal pain, abnormal vaginal discharge, and/or bleeding

III. Scientific Background

Chlamydia

Chlamydia, caused by the bacterium *Chlamydia trachomatis*, is usually an asymptomatic sexually transmitted infection that can be passed to a newborn from an infected mother, potentially resulting in conjunctivitis and/or pneumonia. Symptomatic infections can include cervicitis, pelvic inflammatory disease (PID), and Fitzhugh-Curtis syndrome in women as well as epididymitis, prostatitis, and reactive arthritis triad in men. Both men and women can have proctitis, urethritis, conjunctivitis, pharyngitis, and genital lymphogranuloma venereum as a result of a chlamydial infection. Nucleic acid amplification testing (NAAT) for chlamydia is the gold standard due to high specificity and sensitivity instead of using culture testing, microscopy, or antigen detection (Hsu, 2022). In the U.S. alone, in 2018, over 1.7 million cases of chlamydia were reported to the CDC, but the CDC estimates that 2.86 million chlamydial infections occur annually (CDC, 2021a). This under-reporting is due to individuals who are asymptomatic and, therefore, do not seek treatment. Highest prevalence occurs among men who have sex with men (MSM) and young people. "It is estimated that 1 in 20 sexually active young women aged 14-24 years has chlamydia" (CDC, 2021a).

Mycoplasma genitalium (Mgen) is a sexually transmitted infection that is strongly associated with urethritis symptoms, similar to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Goldstein et al., 2021). *Mgen* can infect the uterus, urethra, or rectum, and causes infections in all genders. In men, common symptoms of *Mgen*-urethritis include: dysuria, urethral pruritus, and purulent or mucopurulent urethral discharge. In women, common symptoms of Mgen cervicitis include: vaginal discharge, vaginal itching, dysuria, and pelvic discomfort. The prevalence of *Mgen* in the United States is estimated to be 1.7% among people aged 14 to 59 years. However, the prevalence of *Mgen* in clinical-based populations are higher; a multicenter study around diverse geographic regions of the United States found the prevalence of *Mgen* to be 10.3% in people seeking care (CDC, 2022c).

Gonorrhea

Gonorrhea is a sexually transmitted infection caused by the bacterium *Neisseria gonorrhoeae*. A gonorrheal infection can cause many of the same complications as chlamydia, including PID, cervicitis, and Fitzhugh-Curtis syndrome in women and epididymitis in men. Urethritis, pharyngitis, and proctitis can also occur; in fact, "*N. gonorrhoeae* can be isolated from the urethra in up to 90 percent of women with gonococcal cervicitis" (Ghanem, 2022). Like chlamydia, if left untreated, gonorrhea can be spread from

mother to newborn, resulting in conjunctivitis. NAAT is the best method to diagnose gonorrhea, but culture testing is still used to determine antimicrobial susceptibility due to an increase in antibiotic resistance (Unemo, 2020). In 2016, the CDC reported an 18.5% increase since 2015 in the number of cases of gonorrhea reported in the United States (CDC, 2023c). The CDC also reported 583,405 new cases of gonorrhea in the United States in 2018 (CDC, 2021d).

Syphilis

Syphilis is caused by the bacterium *Treponema pallidum*, and it progresses, if left untreated, through various stages—primary, secondary, early-latent, late-latent, and late-stage syphilis—until infecting the central nervous system. "Syphilis infection is associated with HIV infection and increases the risk for acquiring or spreading HIV" (Cantor et al., 2016). Worldwide, the median rates of infection in males and females were 17.7 cases per 100,000 and 17.2 cases per 100,000, respectively, according to the World Health Organization. The U.S. has reported an increase in the rate of syphilis between 2000 and 2016, and approximately 90% of the new cases of primary and secondary syphilis during this period occurred in men with 81% occurring in men who have sex with men (MSM). Of concern, there has also been an increased number of cases of syphilis in women. In 2018, 1306 cases of congenital syphilis were reported. This included 78 syphilitic stillbirths and 16 infant deaths. (Hicks & Clement, 2021a).

Similar to other STIs, syphilis is often asymptomatic. For symptomatic syphilis, the signs and symptoms can vary, depending on the stage of disease. Primary syphilis can have a characteristic chancre, a skin lesion, that is usually painless and often heals even in the absence of treatment. Secondary syphilis occurs weeks to months later and can be manifested by typical immunologic responses, such as fever, lethargy, and so on; adenopathy; rash; alopecia; hepatitis; gastrointestinal abnormalities; and even early symptoms of neurological infection, if left untreated. Later stages of syphilis can include cardiovascular abnormalities and progression of neurological syphilitic infection. Asymptomatic, latent syphilis can also occur; moreover, "pregnant women with latent syphilis can transmit *T. pallidum* to their fetus for up to four years after acquisition" (Hicks & Clement, 2022a).

The standard protocol for diagnosing a syphilis infection is to use a two-tiered serological testing algorithm of treponemal testing and nontreponemal testing. Treponemal testing is typically more complex than the latter, and they both rely upon the detection of specific treponemal antigens using enzyme immunoassay (EIA), particle agglutination assay, fluorescence, or chemiluminescence immunoassay (CIA). Nontreponemal testing methods, including the rapid plasma reagin test (RPR) and the venereal disease research laboratory (VDRL) test, "are based upon the reactivity of serum from infected patients to a cardiolipin-cholesterol-lecithin antigen" (Hicks & Clement, 2022b). Rapid serological testing using darkfield microscopy is not as universally used due to complexity and cost. NAAT has not been FDA-approved at this time and is not typically performed for genital syphilis. "There is no internationally approved PCR for *T. pallidum* and accordingly, it is crucial to select a strictly validated method and always use it with appropriate quality controls" (Janier et al., 2014).

Herpes Simplex Virus (HSV)

Herpes Simplex Virus-2 (HSV-2) is the common cause of most of genital herpes simplex infections worldwide with the CDC estimating that 50 million people in the U.S. were infected with HSV-2 in 2015 (Workowski & Bolan, 2015). More than 770,000 people in the U.S. are infected each year with genital herpes; moreover, HSV-1 genital herpes has increased in recent years. This trend is believed to be due to a decline in childhood oral HSV-1 infections that in the past increased immune resistance to genital HSV-1 infections (CDC, 2021b). Primary genital herpes infections can present with genital ulcers as well as other immunological responses, such as fever and lymphadenopathy; however, for some people, a primary genital herpes infection is asymptomatic. Nonprimary infections occur when a patient acquires HSV-1 with pre-existing HSV-2 antibodies or vice versa. Recurrent infections can be either symptomatic or asymptomatic, which can be referred as subclinical. A minority of HSV-positive patients can also present with meningitis and/or proctitis (Albrecht, 2020). Vertical transmission from mother to newborn can occur during delivery, especially if the mother acquires a primary infection near the end of the pregnancy. This vertical transmission can occur even if the mother is asymptomatic (Riley & Wald, 2022). Diagnosis of genital herpes infection can be performed by viral culture, NAAT, and serological testing. "Cell culture and PCR-based testing are the preferred tests for a patient presenting with active lesions, although PCR-based testing has the greatest overall sensitivity and specificity" (Albrecht, 2022).

Human Papillomavirus (HPV)

Anogenital HPV infection is the most common STI worldwide with an estimation that "at least 80 percent of sexually active women and men are exposed to HPV once in their lifetime. However, many experts believe that virtually all sexually active adults have been infected by HPV…"(Palefsky, 2022). This is due to the large number of different types of HPV known to infect the genital tract—at least 40 characterized to date—and the transitory nature of HPV infections. HPV is associated with a variety of cancers, including anal, penile, vulvar, vaginal, and oropharyngeal cancer; moreover, the carcinogenic effect of an HPV infection can be years after the initial diagnosis of HPV. Multiple HPV vaccinations have 12 (CDC, 2022a). HPV can be detected from swab samples and can be included in many routine cervical exams. High-risk oncogenic HPV testing is commercially available (Feldman & Crum, 2022).

HIV Preexposure Prophylaxis (PrEP)

An estimated 1.1 million people in the United States currently live with human immunodeficiency virus (HIV). HIV is a virus that, while treatable, does not have a cure and results in serious health consequences that may include acquiring AIDs (acquired immune deficiency syndrome). In the 2019 issue of JAMA, the US Preventive Services Task Force updated guidelines on recommendations for HIV screening and preventive services. The USPSTF reviewed the evidence regarding Preexposure prophylaxis (PrEP), which is the use of antiretroviral medication to prevent HIV infection ang provided a grade A recommendation for PrEP in certain circumstances (CDC, 2022d; USPSTF, 2019). The USPSTF determined that PrEP is "of substantial benefit in decreasing the risk of HIV infection in persons at high risk of HIV acquisition" (USPSTF, 2019). As a preventive medication, PrEP involves a single treatment taken orally with "combined tenofovir disoproxil fumarate and emtricitabine," or tenofovir disoproxil fumarate alone, which can be considered as an alternative regimen (USPSTF, 2019). In addition, adherence to PrEP is "highly associated with its efficacy in preventing the acquisition of HIV infection; thus, adherence to PrEP is central in realizing its benefit." Overall, the guidance is to provide PrEP with antiretroviral therapy to persons at high risk of HIV acquisition (USPSTF, 2019).

Diagnostic Testing of Common Sexually Transmitted Infections, continued

To determine status for PrEP provision, the CDC recommends antigen/antibody testing to confirm that patients do not currently have HIV infection. At a minimum providers should test to confirm a negative antibody result within a week before initiating (or re-initiating) PrEP regimens (CDC, 2022d). There are a few ways to accomplish HIV testing: "(1) drawing blood and sending the specimen to a laboratory for testing or (2) performing a rapid, point-of-care FDA-approved fingerstick blood test. Oral rapid tests should not be used to screen for HIV infection when considering PrEP use because they can be less sensitive than blood tests" (CDC, 2022d).

The PrEP regimen may cause decreases in renal function. Usually, these are of small or limited clinical significance, but occasional cases of acute renal failure have been documented. The CDC guidance indicates that all patients who are considered for PrEP should have renal function assessed during the beginning of treatment. Other screenings recommended before PrEP initiation include a screening for HBV.

The following table for PrEP testing recommendations for clinicians was compiled by the CDC (CDC, 2022d):

Provide the following services:	Screening tests/samples
At 3 months after PrEP initiation:	• Test for HIV. • Measure serum creatinine and estimate creatinine clearance. • Provide medication adherence and behavioral risk reduction support. • Additionally, for o MSM: screen for bacterial STIs*; o Women with reproductive potential: test for pregnancy; and o PWID: assess access to sterile needles/syringes and to drug treatment services.
Every 3 months after the first 3-month follow-up	• Test for HIV. • Provide medication adherence and behavioral risk reduction support. • Additionally, for o MSM: screen for bacterial STIs*; o Women with reproductive potential: test for pregnancy; and o PWID: assess access to sterile needles/syringes and to substance use disorder treatment services.
Every 6 months after the first 3-month follow-up	• Measure serum creatinine and estimate creatinine clearance. • For all sexually active patients: Screen for bacterial STIs*.

*Nucleic Acid Amplification Test (NAAT) to screen for gonorrhea and chlamydia based on anatomic site of exposure; blood test for syphilis.

Proprietary Testing

BD Onclarity HPV Assay

The BD Onclarity HPV Assay, a qualitative in vitro assay of cervical swabs using PCR (i.e., a nucleic acid amplification test or NAAT), is offered by Becton, Dickinson and Company and is approved by the FDA. This test specifically identifies types 16, 18 and 45, while concurrently detecting the other high-risk (HR) HPV types (including 31, 51, 52, 33/58, 35/39/68, and 56/59/66). For HR-HPV 31, 51, 52, 33/58, 35/39/68, and 56/59/66, this is "the only FDA-approved assay to individually identify and report these genotype results" (BD, 2020).

Becton, Dickinson and Company note that "the BD Onclarity HPV Assay is indicated: 1) In women 21 years and older with ASC-US (atypical squamous cells of undetermined significance) cervical cytology test results, the BD Onclarity HPV Assay can be used to determine the need for referral to colposcopy; 2) In women 21 years and older with ASC-US cervical cytology test results, the BD Onclarity HPV assay can be used to detect high-risk HPV genotypes 16, 18 and 45. This information together with physicians assessment of screening history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy; 3) In women 30 years and older, the BD Onclarity HPV Assay can be used together with cervical cytology to adjunctively screen to detect high risk HPV types. This information, together with the physicians assessment of screening history, other factors, and professional guidelines, may be used to guide patient management; 4) In women 30 years and older, the BD Onclarity HPV Assay can be used to detect high-risk HPV genotypes 16, 18 and 45. This information, together with the physicians assessment of screening history, other factors, and professional guidelines, may be used to guide patient management; and 5) In women 25 years and older, the BD Onclarity HPV Assay can be used as a first-line primary cervical cancer screening test to detect high risk HPV, including 16 and 18. Women who test negative for the high risk HPV types by the BD Onclarity HPV Assay should be followed up in accordance with the physicians assessment of screening and medical history, other risk factors, and professional guidelines. Women who test positive for HPV genotypes 16 and/or 18 by the BD Onclarity HPV Assay should be referred to colposcopy. Women who test high risk HPV positive and 16 and 18 negative by the BD Onclarity HPV Assay (12 other HR HPV Positive) should be evaluated by cervical cytology to determine the need for referral to colposcopy (FDA, 2021)."

Cepheid Xpert® CT/NG

Cepheid offers the Cepheid Xpert® CT/NG test, an FDA approved nucleic acid amplification test to detect *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) using urogenital specimens and extragenital specimens (pharynx and rectum))(FDA, 2012a, 2019a). It is performed using the GeneXpert® Instrument Systems with a qualitative *in vitro* real-time PCR "for the automated detection and differentiation of genomic DNA from Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (NG)" (FDA, 2012b, 2019b) and is stated to provide results for up to 96 specimens in approximately 90 minutes (Cepheid, 2022b). The assay may be used to "test the following specimens from asymptomatic and symptomatic individuals: female and male urine, patient-collected vaginal swabs (collected in a clinical

setting), clinician-collected endocervical swabs, and female and male pharyngeal and rectal swabs" (Cepheid, 2022b).

The test has varying sensitivities and specificities based on the sample obtained and for which disease the assay is testing for. They are listed below (Cepheid, 2022a):

Swabs		CT	NG
Patient Collected Vaginal Swabs:	Sensitivity	99.5%	100%
	Specificity	99.1%	99.9%
Endocervical Swabs:	Sensitivity	96.0%	100%
	Specificity	99.6%	>99.9%
Pharyngeal Swabs:	Sensitivity	95.9%	94.7%
	Specificity	99.7%	98.8%
Rectal Swabs.	Sensitivity	86.0%	91.2%
	Specificity	99.4%	99.6%

Abbott Alinity™ m STI Assay

Abbott offers the Alinity™ m STI AMP Kit. The test is "an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the direct, qualitative detection and differentiation of RNA from Chlamydia trachomatis (CT), Trichomonas vaginalis (TV), Mycoplasma genitalium (MG), and DNA from Neisseria gonorrhoeae (NG)." The test is a four in one multiplex assay that detects four reactions. The first result should appear in under 115 minutes. Abbott reports a sensitivity of 100% for all analytes and specificity with "no cross-activity observed with 148 organisms." The assay may be used to test the following specimens: "endocervical swab specimens, clinician-collected vaginal swab specimens, self-collected vaginal swab specimens (in a clinical setting), gynecological specimens collected in ThinPrep PreservCyt solution, female urine, and male urine" (Abbott, 2023).

Goldstein et al. (2021) performed an international, multicenter study to evaluate accuracy, reproducibility, and clinical performance of the Alinity™ m STI assay. The Alinity™ m STI assay was compared with commonly used STI assays. "The Alinity m STI assay identified accurately and precisely single and mixed pathogens from an analytical panel of specimens" and had "high overall agreement rates with comparator STI assays" (Goldstein et al., 2021).

Analytical Validity

A 2005 study by Cook and colleagues (Cook et al., 2005) reviewed the validity of NAAT for chlamydia and gonorrhea from urine samples as compared to swabs obtained directly from either the cervix or urethra. They reviewed 29 different studies and only included studies using collections of samples obtained from two anatomic sites. Each test required either a secondary culture confirmation or a secondary NAAT-based confirmation. Over 20,000 different patients were included in the pooled study, and three different NAAT assays were monitored—polymerase chain reaction (PCR), transcription-mediated amplification (TMA), and strand displacement amplification (SDA). "The pooled study specificities of each of the 3 assays exceeded 97% when urine samples were tested, for both chlamydial infection and gonorrhea and in both

men and women." The use of PCR for gonorrheal testing, though, from female urine samples had only 55.6% specificity. The authors concluded the following: "Results of nucleic acid amplification tests for *C. trachomatis* on urine samples are nearly identical to those obtained on samples collected directly from the cervix or urethra. Although all 3 assays can also be used to test for *N. gonorrhoeae*, the sensitivity of the polymerase chain reaction assay in women is too low to recommend its routine use to test for gonorrhea in urine specimens (Cook et al., 2005)."

Due to an increase in demand for enzyme immunoassay-based testing of syphilis, Wong et al. (2011) evaluated the validity of such testing—using the Trep-Sure EIA test—to that of the documented Venereal Disease Research Laboratory (VDRL) test and Treponema pallidum particle agglutination (TPPA) assay. Their research included 674 samples. The EIA-based test had a sensitivity of 98.0% and a specificity of 98.6% (Cantor et al., 2016). The authors conclude that "an IgM/IgG sensitive EIA would be an effective alternative to VDRL for syphilis screening" (Wong et al., 2011). An earlier study using another EIA-based assay, the Trep-Check IgG EIA test, conducted at the National Microbiology Laboratory of Canada (Tsang et al., 2007) did not report as positive results as the Wong study. This research consisted of 604 samples submitted from local or provincial hospitals for confirmation of local testing. Their findings were that the Trep-Check IgG EIA had a sensitivity of 85.3% and specificity of 95.6%, but they also report a positive predictive value of 53.7% (Tsang et al., 2007) as compared to the positive predictive value of 98.4% of the Trep-Sure EIA test (Cantor et al., 2016; Wong et al., 2011). These results can be compared to the published results of the accuracy of the TPPA assay of 87.1% sensitivity, 100% specificity, and 100% positive predictive value—albeit in a smaller sample size (n = 198) (Cantor et al., 2016; Juarez-Figueroa et al., 2007).

The US Preventive Services Task Force (USPSTF) conducted a systematic review of the use of serologic screening for genital herpes and published their findings in 2016 (Feltner et al., 2016). Their extensive review consisted of 17 different studies, ranging from 24 to 3,290 participants, in 19 different publications. Reviewing only the serological testing of HSV-2, they note that the "pooled estimates of sensitivity and specificity of the most commonly used test at the manufacturer's cutpoint were 99% (95% CI, 97%-100%) and 81% (95% CI, 68%-98%), respectively." However, they also note that "use of this test at the manufacturer's cutpoint in a population of 100 000 with a prevalence of HSV-2 of 16% (the seroprevalence in US adults with unknown symptom status) would result in 15 840 true-positive results and 15,960 false-positive results (positive predictive value, 50%)." They note the potential psychosocial harm due to false-positive results. The authors conclude, "Serologic screening for genital herpes is associate with a high rate of false-positive test results and psychosocial harms" (Feltner et al., 2016).

In 2021, the US Preventive Services Task Force issued a brief update on genital herpes simplex diagnostics. Their assessment found that viral culture continues to be the gold standard for HSV infections. For central nervous system infections of HSV, PCR continues to be the gold standard, because of the assay's sensitivity of 80% to 90% for lesion specimens. They also indicated that serological tests are used to detect previous infections of herpes simplex in asymptomatic patients, specifying the Western blot assay as the most validated method. In addition, they noted: "two type-specific glycoprotein G serological tests are commercially available in the United States. Sensitivity and specificity of these tests are comparable to the Western blot assay" (Glass, 2021).The ATHENA study conducted in 2008-2009 and published in Lancet in 2011 consisted of more than 40,000 women in the U.S. aged 25 or over in 61 different clinical centers. The goal was to assess high-risk HPV16 and HPV18 testing versus traditional methods. Their results show

that "in women who had colposcopy, the Cobas HPV test was more sensitive than liquid-based cytology for detection of CIN3 [cervical intraepithelial neoplasia grade 3] or worse" with 92.0% versus 53.3% for liquid cytology. "Addition of liquid-based cytology to HPV testing increased sensitivity for CIN3 or worse to 96.7%...but increased the number of screen positives by 35.2%." The authors conclude, "HPV testing with separate HPV16 and HPV18 detection could provide an alternative, more sensitive, and efficient strategy for cervical cancer screening than do methods based solely on cytology (Castle et al., 2011)." Guenat and colleagues report a coefficient of variation of less than 8% for repeatability and reproducibility when using the Novaprep HQ+ medium in liquid-based cytology for HPV (Guenat et al., 2016). Another study comparing the validity of using urine samples in comparison with cervical samples for monitoring HPV in women over the age of 30 shows that the sensitivity of the urine testing varies considerably depending on the NAAT assay used. The multiplex type-specific PCR (E7-MPG) assay had a sensitivity of 80% and specificity of only 61% whereas the GP5+/6+ PCR assay resulted in 58% and 89%, respectively, for sensitivity and specificity as compared to the gold standard cervical swabs (Tshomo et al., 2017).

A study by Golden et al. (2019) compared the sensitivity of syphilis serological testing using the rapid plasma reagin (RPR) test and an experimental 23S rRNA *Treponema pallidum* real-time transcription-mediated amplification (TMA) assay. This study included 545 men who have sex with men (MSM); a total of 506 pharyngeal specimens and 410 rectal specimens were provided for this study. Twenty-two men were diagnosed with syphilis based on serological testing results; further, two more men were diagnosed based on TMA testing results. The authors report that "At least 1 specimen was TMA positive for 12 of 24 men with syphilis (sensitivity, 50% [95% confidence interval [CI], 29 to 71%]). RPR testing and clinical diagnosis were 92% sensitive (95% CI, 73 to 99%) in identifying infected men" (Golden et al., 2019). A combinatory approach of mucosal TMA testing and serological testing may improve the sensitivity of syphilis screening.

Pham et al. (2020) reported on a new prototype POCT based on detecting IgA antibodies for *Treponema pallidum* (TP-IgA), which is a new biomarker for active syphilis. Using "458 pre-characterised stored plasma in China… and 503 venous blood samples collected from pregnant/postpartum in South Africa," the performance of the POCT was compared against TPHA and RPR tests. In the sub-study group from China, the index test had a sensitivity of 96.1% (95% confidence interval 91.7%-98.5%) and specificity of 84.7% (95% confidence interval 80.1%-88.6%) for "identification of active syphilis," (TPHA positive, RPR positive) and identified 71% samples of past-treated syphilis, defined as a TPHA positive but RPR negative test. In the sub-study group from South Africa, the index test had a 100% sensitivity (95% confidence interval 59%-100%) for active syphilis, and "correctly identified all nine women with past syphilis." The researchers cite that in comparison to other POCTs on the market, this new test can "identify past syphilis whilst maintaining a high sensitivity for active syphilis infections," and "support[s] the global effort in prevention of mother to child transmission and elimination of congenital syphilis in settings where laboratory capacity is limited" (Pham et al., 2020).

In 2019, Bristow et al. compared the use of the Xpert® CT/NG test on extragenital samples to the already FDA-approved APTIMA transcription mediated amplification Combo 2 assay. They found the Xpert® CT/NG test performed similarly, but with a faster turnaround time and increased potential for same-day treatment. Their results demonstrated that "the pooled positive and negative percent agreement for detection of CT in rectal specimens was 89.72% (95% CI: 84.97%, 93.64%) and 99.23% (95% CI: 98.74%, 99.60%), and in pharyngeal specimens, they were 89.96% (95% CI: 66.38%, 99.72%) and 99.62% (95% CI:

98.95%, 99.95%) respectively. For NG detection in rectal specimens, the pooled positive and negative per cent agreement was 92.75% (95% CI: 87.91%, 96.46%) and 99.75% (95% CI: 99.46%, 99.93%), and in pharyngeal specimens, they were 92.51% (95% CI: 85.84%, 97.18%) and 98.56% (95% CI: 97.69%, 99.23%) respectively" (Bristow et al., 2019).

A separate study done earlier by Cosentino et al. (2017) also compared APTIMA's transcription mediated Combo 2 assay with the Xpert® CT/NG assay and found that "For *C. trachomatis*, neither system was > 95% sensitive from the rectum, though both were > 99.5% specific. For *N. gonorrhoeae*, Xpert had higher sensitivity than Aptima, but with more false positives from pharyngeal samples."

Clinical Validity and Utility

A 2017 review of point-of-care tests (POCTs) versus near-patient NAAT for chlamydia reviewed 11 different studies consisting of a combined total of more than 13,000 patients. The pooled results show that POCTs have a sensitivity of only 53%, 37%, and 63% for cervical swabs, vaginal swabs, and male urine, respectively, but that the specificity for each ranged from 97-99%. The near-patient NAAT has a sensitivity of > 98% regardless of sample with a specificity of 99.4%. "The systematic reviews show that antigen detection POCTs for CT [*C. trachomatis*], although easy to use, lacked sufficient sensitivity to be recommended as a screening test. A near-patient NAAT shows acceptable performance as a screening or diagnostic test but requires electricity, takes 90 min and is costly (Kelly et al., 2017)." Likewise, a review of five POCTs and one near-patient NAAT for gonorrhea in 2017 show that POTC immunochromatographic tests and optical immunoassays had sensitivities ranging from 12.5% to 70% compared to laboratory NAAT for cervical and vaginal swab samples. The specificities of the near-patient NAATs were > 99.8% with sensitivities > 95% (Guy et al., 2017).

A 2018 review of laboratory testing for *T. pallidum* in Australia (Brischetto et al., 2018) compared the clinical value of PCR testing for syphilis as compared to the traditional serological testing using RPR, agglutination, and/or chemiluminescence immunoassay (CMIA). This review covered all testing at the Australian lab from 2010 to 2017. They show that 19% of PCR results were positive for syphilis with 97% of those patients also showing positive serological results. The *T. pallidum* PCR had a sensitivity of 68% and specificity of 99% as compared to the serology testing sensitivity of 97% and 88% specificity. "Our results show that most patients with positive *T. pallidum* PCR results also had positive syphilis serology. Therefore, *T. pallidum* PCR adds little clinical value over serology for the diagnosis of syphilis in certain clinical settings (Brischetto et al., 2018)." A 2015 Chinese study (Zhiyan et al., 2015) does show that the CMIA screening is not as specific as the TPPA agglutination assay for syphilis with 18 of the 149 CMIA-positive samples being false-positive results.

The 2016 USPSTF review of genital herpes serological testing (Feltner et al., 2016) included a review of the HerpeSelect serological test consisting of the data from ten studies with a combined total of 6537 participants. The pooled, combined results show a sensitivity of 99% and specificity of 81%. Four additional studies they reviewed used the biokit HSV-2 Rapid Test assay. These studies had a combined total of 1512 participants. The sensitivity is considerably lower (84%), but the specificity was higher than the HerpeSelect assay (95%).

A study by Liu and associates (Liu et al., 2014) evaluated the clinical performance of the QuantiVirus HPV E6/E7 mRNA with respect to identifying ≥Grade 2 cervical intraepithelial neoplasia. Approximately 40.3%

of the 335 female patients tested positive for high-risk HPV. They note that "the positivity rate of HPV E6/E7 mRNA increased with the severity of cytological and histological evaluation…a high specificity and a low positivity rate of E6/E7 mRNA testing as a triage test in HPV DNA-positive women can be translated into a low referral for colposcopy (Liu et al., 2014)." Another study of the QuantiVirus system in 2017 (Yao et al., 2017) of 404 HPV-positive women show no statistical difference between QuantiVirus and cytological testing in sensitivity, specificity, positive predictive value, and negative predictive value for predicting high-grade squamous intraepithelial lesion (HSIL). "HPV E6/E7 mRNA detection in cervical exfoliated cells shows the same performance as Pap triage for HSIL identification for HPV-positive women. Detection of HPV E6/E7 mRNA may be used as a new triage option for HPV-positive women (Yao et al., 2017)." A review by Arbyn and colleagues concerning the efficacy of repeat cytology versus HPV testing for atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LSIL) demonstrated that the pooled sensitivity of the Hybrid Capture 2 (HC2) assay for the high-risk HPV types was significantly higher than performing repeat cytology (relative sensitivity of 1.27 and 1.23, respectively) for detecting CIN2+ but was significantly lower than repeat cytology for LSIL. "HPV-triage with HC2 can be recommended to triage women with ASCUS because it has higher accuracy…than repeat cytology. When triaging women with LSIL, an HC2 test yields a significantly higher sensitivity, but a significantly lower specificity, compared to repeat cytology. Therefore, practice recommendations for management of women with LSIL should be balanced, taking local circumstances into account (Arbyn et al., 2013)."

A study by Gaydos et al. (2019) showed that, for women in the emergency department (ED), the use of rapid diagnostic tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections can improve clinical management. This randomized clinical trial was composed of 254 women undergoing pelvic examinations for both *C. trachomatis* and *N. gonorrhoeae* testing; the women were split into control and rapid test groups. For the rapid test group, the GeneXpert rapid test was used. The authors report that "Undertreatment for both *C trachomatis* and *N gonorrhoeae* in the ED was 0% for the rapid test group and 43.8% for the control standard-of-care group. Clinicians overtreated 46.5% of uninfected standard-of-care control patients for *C trachomatis* compared with 23.1% of uninfected rapid test patients. For patients uninfected with *N gonorrhoeae*, clinicians overtreated 46.7% of standard-of-care control patients compared with 25.4% of rapid test patients" (Gaydos et al., 2019). These results show that rapid testing of *C. trachomatis* and *N. gonorrhoeae* led to a significant reduction in overtreatment compared to the control group.

IV. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

Anal Carcinoma (NCCN, 2022a): HPV, especially high-risk types HPV-16 and HPV-18, are linked to anal carcinoma. The NCCN refers to a study that detected HPV in 84% of anal carcinoma samples and 0% in rectal cancer samples, and they state that "the prevalence of HPV-16/18 to be 72% in patients with invasive anal cancer." Precursor high-grade anal intraepithelial neoplasia (AIN) "can be identified by cytology, HPV testing, digital rectal examination (DRE), high-resolution anoscopy, and/or biopsy." They also state that "data suggest that HPV- and/or p16-positivity are prognostic for improved OS [overall survival] in patients with anal carcinoma." For females, the NCCN also recommends a gynecologic examination, including cervical cancer screening, due to the link between HPV and anal carcinoma.

Diagnostic Testing of Common Sexually Transmitted Infections, continued

Cervical Cancer (NCCN, 2023a): "Persistent human papillomavirus (HPV) infection is the most important factor in the development of cervical cancer. The incidence of cervical cancer appears to be related to the prevalence of HPV in the population…. Screening methods using HPV testing may increase detection of adenocarcinoma." The NCCN lists chronic, persistent HPV infection along with persistently abnormal Pap tests as criteria to be considered for women contemplating hysterectomy after the completion of childbearing.

Head and Neck Cancers (NCCN, 2022b): The NCCN in the Head and Neck Cancers guidelines now specifically states, "Tumor human papillomavirus (HPV) testing by p16 immunohistochemistry (IHC) required" in their workup for cancer of the oropharynx because the p16 status dictates the treatment options to be considered (per the ORPH-1 workup). This version of the guidelines also includes a page on the "Principles of P16 Testing for HPV-Mediated Oropharyngeal Cancer" where they state the following:

- "P16 expression is highly correlated with HPV status and prognosis and is widely available."
- "A few HPV testing options are available for use in the clinical setting. Expression of p16 as detected by IHC is a widely available surrogate biomarker that has very good agreement with HPV status as determined by the gold standard of HPV E6/E7 mRNA expression. Other tests include HPV detection through PCR and in situ hybridization (ISH).
- "Sensitivity of IHC staining for p16 and PCR-based assay is high, although specificity is highest for ISH."
- "Due to variations in sensitivity and specificity values of testing options, multiple methods may be used in combination for HPV detection, but HPV detection through PCR and ISH may provide additional sensitivity for the former and specificity for the latter in the case of an equivocal p16 or unclear clinical scenario."
- "Sufficient pathologic material for HPV testing can be obtained through FNA."
- "A small proportion of tumors at non-oropharyngeal sites (eg, paranasal sinus, oral cavity, larynx) are HPV-related. However, given the small proportion and lack of consistent evidence in support of prognostic significance, routine HPV testing or p16 [testing] of non-oropharyngeal cancers is not recommended."
- "Guidelines for testing are available from the College of American Pathologists."

Occult Primary Cancers (NCCN, 2023d): The NCCN now lists HPV to be tested for Occult Primary cancers. The NCCN also states that for squamous cell carcinoma with a clinical presentation in the head and neck nodes, "Check results of p 16 immunohistochemistry/HPV in situ hybridization and EBV in situ hybridization; positive results can help localize primary site." Further, the guidelines note that HPV can be used as a potential immunohistochemistry marker for unknown primary cancers, including tumors identified in the cervix, vulva, vagina, penis, anal, oropharynx; a nuclear (DNA ISH) or nuclear/cytoplasmic (RNA ISH) staining pattern is recommended (NCCN, 2021c).

Penile Cancer (NCCN, 2023b): "Overall, approximately 45% to 80% of penile cancers are related to HPV, with a strong correlation with types 16, 6 and 18." Discerning whether a penile cancer lesion is infected with HPV is important for laser ablation therapy as noted in the section titled "Principles of Penile Organ-Sparing Approaches."

Vulvar Cancer (NCCN, 2023c): "Risk factors for the development of vulvar neoplasia include increasing age, infection with human papillomavirus (HPV), cigarette smoking, inflammatory conditions affecting the vulva, and immunodeficiency…. Usual-type VIN [vulvar intraepithelial neoplasia] was linked to persistent infection with carcinogenic strains of HPV, while differentiated VIN was commonly associated with vulvar dermatologic conditions such as lichen sclerosus. In 2015, the ISVVD updated the description to three classes of vulvar lesions: 1) low-grade squamous intraepithelial lesion (LSIL) due to flat condyloma or HPV effect; 2) high-grade squamous intraepithelial lesions (HSIL, formerly considered usual-type VIN); and 3) differentiated VIN." The NCCN notes that 80-90% of HSIL cases have HPV infections, and that between 30%-69% of all vulvar cancers are believed to be "attributable to HPV infection." In the "Diagnosis and Workup" section, they state, "Appropriate patients should receive smoking cessation counseling and HPV testing." The guidelines also note for the surveillance of vulvar cancer: "cervical/vaginal cytology screening as indicated for the detection of lower genital tract neoplasia (may include HPV testing)" (NCCN, 2021b).

U.S. Preventive Services Task Force (USPSTF)

Screening for Chlamydia and Gonorrhea (Davidson et al., 2021): The USPSTF recommends (Grade B) to screen for chlamydia and gonorrhea in "sexually active females aged 24 years or younger and in women 25 years or older who are at increased risk for infection." They also conclude (an "I" statement) "that the current evidence is insufficient to assess the balance of benefits and harms of screening for chlamydia and gonorrhea in men." Besides age, "women 25 years or older are at increased risk for infection if they have a new sex partner, more than 1 sex partner, a sex partner with concurrent partners, or a sex partner who has an STI; practice inconsistent condom use when not in a mutually monogamous relationship; or have a previous or coexisting STI. Exchanging sex for money or drugs and history of incarceration also are associated with increased risk." They clearly state that both chlamydia and gonorrhea should be tested using NAATs.

Screening for Oral Cancer (Moyer, 2014): Given the link between HPV infection and oral cancers, the USPSTF released their findings concerning the screening of asymptomatic patients. "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for oral cancer in asymptomatic adults." They also state the following: "Although there is interest in screening for oral HPV infection, medical and dental organizations do not recommend it. Currently, no screening test for oral HPV infection has been approved by the U.S. Food and Drug Administration (FDA). Evaluating the accuracy of tests that detect oral HPV infection is a potentially promising area of research (Moyer, 2014)."

Serological Screening for Genital Herpes (Feltner et al., 2016): HSV-2 is the primary causative agent of genital herpes, and HSV-2 infection during pregnancy can cause fetal morbidity and mortality. Due to its prevalence in the U.S. and the possible consequences of a genital herpes infection, the USPSTF researched the validity and practicality of HSV-2 screening in asymptomatic patients. They conclude that "serologic screening for genital herpes is associated with a high rate of false-positive test results and potential psychosocial harms. Evidence from RCTs [randomized clinical trials] does not establish whether preventive antiviral medication for asymptomatic HSV-2 infection has benefit." Overall, the USPSTF "recommends

against routine serologic screening for genital herpes simplex virus (HSV) infection in asymptomatic adolescents and adults, including those who are pregnant."

Screening for Syphilis (Cantor et al., 2016): Previously, in 2004, the USPSTF "recommended routine screening for syphilis in asymptomatic men and nonpregnant women at increased risk of infection (A recommendation) and recommended against routine screening for those not at increased risk (D recommendation)." The previous study did not address the frequency of repeat testing. The current 2016 study adds to the previous recommendations. "Screening HIV-positive men or MSM for syphilis every 3-months is associated with improved syphilis detection. Treponemal or nontreponemal tests are accurate screening tests but require confirmation. Research is needed on the effect of screening on clinical outcomes; effective screening strategies, including reverse sequence screening, in various patient populations; and harms of screening."

Centers for Disease Control and Prevention (CDC)

Diseases Characterized by Genital, Anal, or Perianal Ulcers: "…all persons who have genital, anal, or perianal ulcers should be evaluated; … Specific evaluation of genital, anal, or perianal ulcers includes syphilis serology tests and darkfield examination from lesion exudate or tissue, or NAAT if available; NAAT or culture for genital herpes type 1 or 2; and serologic testing for type-specific HSV antibody. In settings where chancroid is prevalent, a NAAT or culture for Haemophilus ducreyi should be performed.." Later, in the section specifically focused on genital HSV infections, the CDC states, "Both type-specific virologic and type-specific serologic tests for HSV should be available in clinical settings that provide care to persons with or at risk for STIs." They stress that the patient's prognosis does depend on the type of HSV infection, especially since "recurrences and subclinical shedding are much more frequent for genital HSV-2 infection than for genital HSV-1 infection." Regarding testing, "HSV NAAT assays are the most sensitive tests because they detect HSV from genital ulcers or other mucocutaneous lesions; these tests are increasingly available"(CDC, 2021f). NAATs are more sensitive than viral culture testing. On the CDC's detailed fact sheet about genital herpes, they state, "Routine serologic HSV screening of pregnant women is not recommended" (CDC, 2021a).

In guidance on serology, the CDC states in 2021 that "type-specific HSV-2 serologic assays for diagnosing HSV-2 are useful in the following scenarios: recurrent or atypical genital symptoms or lesions with a negative HSV PCR or culture result, clinical diagnosis of genital herpes without laboratory confirmation, and a patient's partner has genital herpes. HSV-2 serologic screening among the general population is not recommended. Patients who are at higher risk for infection (e.g., those presenting for an STI evaluation, especially for persons with ≥10 lifetime sex partners, and persons with HIV infection) might need to be assessed for a history of genital herpes symptoms, followed by type-specific HSV serologic assays to diagnose genital herpes for those with genital symptoms"(CDC, 2021d).

Syphilis: Darkfield examinations and molecular tests for detecting *T. pallidum* lesion cells, fluid, or tissue are the gold standard methods for diagnosing early syphilis and congenital syphilis. According to the CDC, "Although no *T. pallidum* direct detection molecular NAATs are commercially available, certain laboratories provide locally developed and validated PCR tests for detecting *T. pallidum* DNA. A presumptive diagnosis of syphilis requires use of two laboratory serologic tests: a nontreponemal test

(i.e., Venereal Disease Research Laboratory [VDRL] or rapid plasma reagin [RPR] test) and a treponemal test (i.e., the T. pallidum passive particle agglutination [TP-PA] assay, various EIAs, chemiluminescence immunoassays [CIAs] and immunoblots, or rapid treponemal assays) … Use of only one type of serologic test (nontreponemal or treponemal) is insufficient for diagnosis and can result in false-negative results among persons tested during primary syphilis and false-positive results among persons without syphilis or previously treated syphilis." If a patient shows signs and symptoms of neurosyphilis, including "cranial nerve dysfunction, auditory or ophthalmic abnormalities, meningitis, stroke, acute or chronic altered mental status, and loss of vibration sense," further testing is required-CSF cell count or protein and a reactive CSF-VDRL (CDC, 2021d).

The CDC states the signs and symptoms of neurosyphilis can include severe headache, trouble with muscle movements, muscle weakness or paralysis (not being able to move certain parts of the body), numbness, and changes in mental status (trouble focusing, confusion, personality change) and/or dementia (problems with memory, thinking, and/or making decisions). The CDC states that signs and symptoms of ocular syphilis can include eye pain or redness, floating spots in the field of vision ("floaters"), sensitivity to light, and changes in vision (blurry vision or even blindness). Lastly, the CDC states that signs and symptoms of otosyphilis may include hearing loss, ringing, buzzing, roaring, or hissing in the ears ("tinnitus"), balance difficulties, and dizziness or vertigo" (CDC, 2023d).

"Patients who receive a diagnosis of syphilis and have neurologic, ocular, and/or otologic symptoms should be evaluated for neurosyphilis, ocular syphilis, or otosyphilis according to their clinical presentation. Patients who have syphilis and symptoms or signs suggestive of neurologic disease (e.g., cranial nerve dysfunction, meningitis, stroke, acute or chronic altered mental status, or motor or sensory deficits) should have an evaluation that includes CSF analysis before treatment. Patients with syphilis who have symptoms or signs of ocular syphilis (e.g., uveitis, iritis, neuroretinitis, or optic neuritis) should have a full ocular slit-lamp and ophthalmologic examination, including a thorough cranial nerve evaluation; if cranial nerve dysfunction is present, CSF examination is indicated" (CDC, 2021c). The CDC also recommends that, prior to donating, prospective hematopoietic stem cell transplant donors should be tested for syphilis (Dykewicz et al., 2000).

Chlamydial Infections: "Annual screening of all sexually active women aged <25 years is recommended, as is screening of older women at increased risk for infection (e.g., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection…screening of sexually active young men should be considered in clinical settings with a high prevalence of chlamydia (e.g., adolescent clinics, correctional facilities, or STD specialty clinics) or for populations with a high burden of infection (e.g., MSM)" (CDC, 2021f).

NAAT testing of first-catch urine or swab specimens is recommended. In the diagnostic considerations section of Chlamydial Infections, the CDC does not address any differences between symptomatic or asymptomatic screening, and they do not mention any specific diagnostic considerations of patients showing signs or symptoms of a chlamydial infection. In the 2014 CDC guide for laboratory testing of chlamydia and gonorrhea, they, too, recommend using NAATs and not the older nonculture or non-NAAT testing methods. For extragenital infections such as rectal and oropharyngeal infections, the CDC

recommends testing at the anatomic exposure site. NAATs demonstrate improved sensitivity and specificity in comparison to culture for extragenital infection. In addition, newly available molecular point-of-care (POC) tests for asymptomatic persons can help with faster, more efficient treatment. With symptomatic cases these POC tests can also "optimize treatment by limiting unnecessary presumptive treatment at the time of clinical decision-making and improve antimicrobial stewardship. Thus, using a POC test will likely be a cost-effective diagnostic strategy for *C. trachomatis* infection… newer NAAT-based POC tests have promising performance and are becoming commercially available" (CDC, 2021f).

Gonococcal Infections: The CDC recommendation concerning gonococcal screening is similar to that of chlamydia—sexually active women aged <25 years and older women and men in high-risk categories. "Screening for gonorrhea in men and older women who are at low risk for infection is not recommended" (CDC, 2021f). For testing genitourinary infection with N. gonorrhoeae, "culture, NAAT, and POC NAAT, such as GeneXpert (Cepheid), are available." NAAT allows for best testing of genitourinary infection.

For rectal, oropharyngeal, and conjunctival infections, culture is available. The CDC states that "NAATs and POC NAATs allow for the widest variety of FDA-cleared specimen types, including endocervical and vaginal swabs and urine for women, urethral swabs and urine for men, and rectal swabs and pharyngeal swabs for men and women. However, product inserts for each NAAT manufacturer should be consulted carefully because collection methods and specimen types vary. Certain NAATs that have been demonstrated to detect commensal Neisseria species might have comparable low specificity when testing oropharyngeal specimens for N. gonorrhoeae. NAAT sensitivity for detecting N. gonorrhoeae from urogenital and nongenital anatomic sites is superior to culture but varies by NAAT type. NAAT testing of rectal and/or oropharyngeal swab specimens can be performed in certain laboratories that have met CLIA requirements even though the testing methodology has not been FDA-approved"(CDC, 2021f). Follow-up testing post-treatment for urogenital or rectal gonorrhea is not necessary, but NAAT testing should be performed 14 days after treatment for pharyngeal gonorrhea. Vaginitis is the most common symptom of infection in preadolescent girls (Workowski & Bolan, 2015)."

In the 2014 laboratory guide, the CDC states that "*N. gonorrhoeae* culture capacity is still needed for evaluating suspected cases of treatment failure and monitoring antimicrobial susceptibility." They also state, "*C. trachomatis* and *N. gonorrhoeae* culture capacity might still be needed in instances of child sexual assault in boys and extragenital infections in girls" (Papp et al., 2014).

Mycoplasma genitalium Infections: The CDC recommends that men with recurrent nongonococcal urethritis (NGU) should be tested for *M. genitalium* using an FDA-cleared NAAT. The CDC also recommends that women with recurrent cervicitis should be tested for *M. genitalium,* while testing should be considered in women with PID. For both, resistance testing is recommended if testing is available. The CDC notes that screening of asymptomatic "*M. genitalium* infection among women and men or extragenital testing for *M. genitalium* is not recommended. In clinical practice, if testing is unavailable, *M. genitalium* should be suspected in cases of persistent or recurrent urethritis or cervicitis and considered for PID"(CDC, 2021b).

"*M. genitalium* is an extremely slow-growing organism. Culture can take up to 6 months, and technical laboratory capacity is limited to research settings. NAAT for *M. genitalium* is FDA cleared for use with

urine and urethral, penile meatal, endocervical, and vaginal swab samples . . . Molecular tests for macrolide (i.e., azithromycin) or quinolone (i.e., moxifloxacin) resistance markers are not commercially available in the United States. However, molecular assays that incorporate detection of mutations associated with macrolide resistance are under evaluation" (CDC, 2021b).

Human Papillomavirus Infections: Even though testing for oncogenic HPV variants exists, the CDC states, "These tests should not be used for male partners of women with HPV or women aged <25 years, for diagnosis of genital warts, or as a general STI test." For patients showing signs and symptoms of anogenital warts, the CDC states, "HPV testing is not recommended for anogenital wart diagnosis, because test results are not confirmatory and do not guide genital wart management." For cervical screening, "For persons aged 30–65 years, a cytology test every 3 years, an HPV test alone every 5 years, or a cytology test plus an HPV test (cotest) every 5 years is recommended" (CDC, 2021f).

The CDC (2022a) also notes that "Routine screening for women aged 21 to 65 years old can prevent cervical cancer"; further, "There are HPV tests that can be used to screen for cervical cancer. Healthcare providers only use these tests for screening in women aged 30 years and older. HPV tests are not recommended to screen men, adolescents, or women under the age of 30 years."

Finally, the CDC (2022b) states that "there is currently no approved test for HPV in men. CDC does not recommend routine testing (also called 'screening') for HPV in men. CDC also does not recommend routine testing for diseases from HPV before there are signs or symptoms in men. Some healthcare providers offer anal Pap tests to men who may be at greater risk for anal cancer. This includes men with HIV or men who receive anal sex. If you have symptoms and are concerned about cancer, please see a healthcare provider."

International Union Against Sexually Transmitted Infections (IUSTI)

The Management of Anogenital Warts (European): "HPV detection or typing does not influence management and is not recommended. Some practitioners use the acetic acid test to diagnose sub-clinical HPV lesions; its place in diagnosis and management is uncertain" (Gilson et al., 2020) .

The Diagnosis and Treatment of Gonorrhea in Adults (Unemo, 2020) NAATs, bacterial culture, and microscopy can be used in the diagnosis of uncomplicated gonorrhea. "No test offers 100% sensitivity and specificity." They do state (with a grade C recommendation) that microscopy can be used for testing symptomatic men, but it is not recommended for use in asymptomatic men, rectal infection, or endocervical infection due to low sensitivity. Culture testing is the only method to use for determining antimicrobial susceptibility, but culture testing is not as sensitive as NAAT. For NAAT-based point-of-care tests (POCTs), the guideline says: "several NAAT-based POCTs with high sensitivity and specificity are in late development." The IUSTI includes the following list for "Indications for testing" (grade C recommendation):

- Symptoms or signs of urethral discharge in men;
- Vaginal discharge with risk factor for STI (age <30 years, new sexual partner);

Diagnostic Testing of Common Sexually Transmitted Infections, continued

- Mucopurulent cervicitis;
- Persons diagnosed with any other STI;
- Sexual partner of persons with an STI or PID;
- Acute epididymo-orchitis in a male aged <40 years;
- Acute pelvic inflammatory disease;
- When screening young adults (<25 years of age) for sexually transmitted infections;
- When screening individuals with new or multiple recent sexual partners;
- Purulent conjunctivitis in a neonate or adult;
- Mother of a newborn with ophthalmia neonatorum
- Unplanned termination of pregnancy in places or populations of high gonorrhoea prevalence
- When intrauterine interventions are performed in areas of high gonorrhoea prevalence

The Management of Lymphogranuloma Venereum (de Vries et al., 2019): Lymphogranuloma venereum (LGV) is a condition caused by chlamydia. The clinical features can vary, depending on the site of inoculation (genital versus rectum) and can include hemorrhagic proctitis, lymphadenopathy, papule or pustule formation, and buboes. Reactive inflammatory responses or physical signs of in infection may include "constitutional symptoms such as low-grade fever, chills, malaise, myalgia, [and] arthralgia." Regarding a diagnosis of lymphogranuloma venereum (LGV), "a sample tested *C. trachomatis* positive with a commercial nucleic acid amplification test (NAAT) platform should be confirmed with an LGV discriminatory NAAT." Further, "For sensitive and specific detection of LGV genovar (L1, L2 and L3, including subvariant)-specific *C. trachomatis* DNA, laboratories are currently recommended to use a two-step procedure (1,B):

- "A commercially available NAAT is used to detect *C. trachomatis* DNA/RNA in suspected clinical samples. These tests cannot discriminate between LGV and non-LGV genovars. Although no commercially available *C. trachomatis* NAATs are FDA-cleared for extragenital specimens, for several NAATs sufficient evidence supports the use of these tests for the detection of C. trachomatis DNA/RNA also in rectal and pharyngeal *C. trachomatis* infections. Some *C. trachomatis* NAAT are CE-labelled for use on rectal and pharyngeal samples in Europe.
- If *C. trachomatis* DNA/RNA is detected, LGV genovar specific *C. trachomatis* DNA should be detected from the same specimen. There are multiplex NAATs for genital ulcerative disease that detect LGV but these have not yet been appropriately evaluated in the context of rectal LGV. Different in-house or laboratory-developed NAATs have been designed and used. The sensitivities of these NAATs are generally lower than the commercially available *C. trachomatis* screening NAAT (de Vries et al., 2019)."

The Management of Syphilis (Janier et al., 2014; Janier et al., 2020): The three stages (primary, secondary, and tertiary) can be overlapping. Primary syphilis begins with appearance of an ulcer (also known as a chancre), usually in the anogenital region with regional lymphadenopathy. "Any anogenital ulcer should be considered syphilitic unless proven otherwise." The secondary stage is characterized by "multisystem involvement due to bacteriaemia, within the first year but may recur up into the second year after infection" and can include skin rash, generalized lymphadenopathy, arthritis, hepatitis, splenomegaly, and

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kidney dysfunction. Early neurosyphilis can occur in secondary syphilis and can include "meningitis, cranial nerve palsies, auricular and ophthalmic abnormalities (such as uveitis, retinitis, otitis and papillar oedema)." They list the following as conditions of tertiary syphilis:

- "Gummatous syphilis: nodules/plaques or ulcers (skin, mucosae, visceral)"
- "Late neurosyphilis encompasses meningitis, cranial nerve dysfunction, meningovascular syphilis (stroke, myelitis) and parenchymatous neurosyphilis (general paresis, tabes dorsalis)"
- "Cardiovascular syphilis: aortic regurgitation, stenosis of coronary ostia, aortic aneurysm (mainly thoracic)"

The following guidelines were given regarding laboratory testing for *T. pallidum:*

- "Direct detection methods provide definitive diagnosis of syphilis.
- Darkfield examination (DFE) of chancres and erosive cutaneous lesions was the old gold standard method for definitive diagnosis. It gives immediate results. However, the method is labor intensive, subjective, and can result in some false positive and (many) false negative results. Due to the availability of more sensitive and specific tests (specifically the PCR), it is not recommended for routine diagnosis anymore.
- Polymerase chain reaction (PCR) testing is the preferred method particularly but not exclusively for oral and other lesions where contamination with commensal treponemes is likely. It can be performed using tissues, cerebrospinal fluid (CSF) or blood (although insensitive in the latter). There is no internationally approved PCR assay for *T. pallidum* and accordingly, it is crucial to select a strictly validated and quality-assured method and always use it with appropriate quality controls.
- Immunohistochemistry using a polyclonal antibody against *T. pallidum* can be efficient to identify treponemes in skin, mucosal and tissue lesions, but it is not suitable for routine diagnosis.
- Hybridization in tissues is not used for routine diagnosis.
- Warthin-Starry (argentic) staining on tissues is very difficult to perform and of limited value in most cases.
- (Direct fluorescent antibody test is obsolete)
- For molecular epidemiological typing, PCR, PCR-restriction fragment length polymorphism (RFLP) and/or DNA-sequencing (e.g., multilocus sequence typing (MLST) or whole genome sequencing) can be performed on clinical specimens. However, due to the highly conserved genome of *T. pallidum* the discriminatory ability of typing methods is in general low (Janier et al., 2020)"

Primary Screening Test(s)

- "TT [TPHA, MHA-TP, TPPA or EIA/ELISA/CLIA] – a TT-based screening algorithm, using by preference an automatized EIA/ELISA/CLIA, is used in many large, well-resourced European laboratories and is particularly suitable for automated high-throughput screening of asymptomatic populations including blood/plasma donors. The algorithm identifies persons with previous successful treatment of syphilis as well as those with untreated syphilis. It is usually more sensitive in detecting very early syphilis compared to the use of a screening NTT. However, it can also result in a high number of false positive tests (i.e. very low positive predictive value) in low-prevalence populations.

- NTT [RPR or VDRL] – a NTT-based screening algorithm; preferably quantitative (i.e., to detect prozone phenomenon in infectious syphilis), is still recommended in some countries. In this algorithm, only active (Robinson & Canadian Paediatric Society) syphilis is detected, however, it has a lower sensitivity compared to using a TT as primary screening test, and in particular very early syphilis can be missed.
- TT combined with a NTT - this algorithm is particularly useful in cases where the suspicion of very early syphilis is high (recent chancre, contacts of syphilis cases etc.), because in some patients NTT may become reactive before TT (Janier et al., 2020)."

Confirmatory test(s) if any screening test is positive

- "In the case a TT being used alone as a primary screening test, if positive, a confirmatory TT of a different type is of limited value in informing treatment, but a reflex quantitative NTT (reaching at least 1:8 to 1:16 dilution) should be performed in all cases on the same serum (1, B). Although a confirmatory TT may be important for counselling, notification and may have a psychological impact, it has limited impact on treatment.69 In patients with a positive TT, a negative NTT and no suspicion of very early syphilis (no chancre), both tests should be repeated after 1 month (1, D). However, CLIA and EIA used in many European settings have suboptimal specificity, resulting in a low positive predictive value in low prevalence population. 22,49,56 If such tests are used, additionally a reflex confirmatory test by TPHA or TPPA should be performed (1, C).
- In the case a NTT alone is used as a primary screening test, a positive test must be followed by a reflex TT on the same serum. If quantitative NTT was not initially done, the NTT should be repeated quantitatively (1, B).
- In the case both a TT and a NTT are used as primary screening tests such as (EIA/ELISA/CLIA/TPHA/TPPA plus VDRL/RPR), the NTT must be performed quantitatively (if not initially done) in case of positive or discrepant screening tests (1, B).
- The IgG-immunoblot for Treponema pallidum has no added major value to other TT. It is expensive and interpretation of undetermined immunoblot is elusive (1 to 4 bands).

The Management of Chlamydia Trachomatis Infections (Lanjouw et al., 2016): "Appropriate testing of symptomatic and asymptomatic sexually active individual is recommended to identify and treat the *C. trachomatis* infections." With a Grade A recommendation, they recommend using NAATs that identify specific nucleic acid, either DNA or RNA) *of C. trachomatis* "due to their superior sensitivity, specificity, and speed."

The following list contains the indications for laboratory testing as recommended by the IUSTI with a Grade C recommendation (Lanjouw et al., 2016):

Indications for laboratory testing (Level of evidence IV; Grade C recommendation)

- Risk factor(s) for *C. trachomatis* infection and/or other STI (age<25 years, new sexual contact in the last year, more than one partner in the last year);
- Symptoms or signs of urethritis in men;
- Cervical or vaginal discharge with risk factor for STI;
- Acute epididymo-orchitis in a male aged <40 years or with risk factors for STI;

- Acute pelvic pain and/or symptoms or signs of PID;
- Proctitis/proctocolitis according to risk;
- Purulent conjunctivitis in a neonate or adult;
- Atypical neonatal pneumonia;
- Persons diagnosed with other STI;
- Sexual contact of persons with an STI or PID;
- Termination of pregnancy;
- Any intrauterine interventions or manipulations.

The Management of Genital Herpes (Patel et al., 2017): The principle change to the IUSTI guidelines in this recent version is that "HSV DNA detection rather than cell culture is now the gold standard for diagnosis." With a grade C recommendation, "serological testing is not routinely recommended in asymptomatic patients." They note that there are specific groups where it may be useful, including pregnant women, sexual partners of HSV-positive people, those with a history of recurrent or atypical genital disease, and those with first-episode genital herpes whose differentiation may aid in counseling and management (because seroconversion happens typically at 90 days post-infection).

Male Training Center for Family Planning & Reproductive Health (MTC), Office of Population Affairs, Department of Health and Human Services

In general, the MTC recommends at least annual testing for chlamydia, gonorrhea, syphilis, HIV/AIDS, and Hepatitis C for anyone in an at-risk population, including MSM. For syphilis, certain populations require testing at 3-6 month intervals, including those who exchange sex for drugs, commercial sex workers, and young MSM.

The MTC does not recommend screening for pharyngeal chlamydia infections. They do recommend follow-up test three months after initial positive chlamydia test. They recommend using a urine-based NAAT for chlamydia for at-risk male populations under the age of 25, which include MSM, patients at STI clinics, and military personnel (under the age of 30), and inmates entering jails or detention centers (under the age of 30). Men who have had receptive anal intercourse in the preceding year should have a NAAT performed on a rectal swab to check for rectal chlamydial infection.

The MTC recommends using NAAT for gonorrhea testing of at-risk male adolescents and adults, including MSM. "Males with gonorrhea infection should be re-screened for reinfection at 3 months." Annual exams for MSM include screening for urethral infections, pharyngeal infections using NAAT for those "who have had receptive oral intercourse" during the preceding year, and rectal infections using NAAT of rectal swabs for those "who have had receptive anal intercourse" during the preceding year. "More frequent STD screening (i.e., at 3 – 6 month intervals) is indicated for MSM who have multiple or anonymous partners (Marcell & Health, 2014)."

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"For anal warts, no specific testing is recommended to verify the presence or type of HPV as this will not alter management. Anal Pap and/or HPV testing may be of value to identify precancerous anal intraepithelial neoplasia (AIN) in high-risk groups… Although no products are currently licensed for these

[pharyngeal] specimens in Canada, validated NAATs can be used to detect oropharyngeal *N. gonorrhoeae* and *C. trachomatis* infections. Confirmation of positives with culture or a second NAAT should be performed." NAAT can be performed on first-void urine samples from male patients or vaginal swabs or urine samples obtained from female patients. Since NAAT allows for the testing of antimicrobial susceptibility in gonorrheal infections, "depending on the clinical situation, consideration should be given to using both culture and NAAT, especially in symptomatic patients." For oral lesions of suspected HSV, they recommend using NAAT or to obtain fluid for culture. "NAATs approach sensitivities and specificities of 100%, with rapid turn-around of results." For syphilis, "NAATs can be used as a non-serological method for identifying *T. pallidum* in mucosa and skin involve. They are very sensitive and specific. When genital lesions characteristic of early syphilis are present, clear serous fluid may be collected for dark-field microscopy, enabling observation of morphology and movement of the spirochetes for the detection of *T. pallidum* (not reliable for oral or rectal lesions)" (Chernesky et al., 2017).

American Academy of Pediatrics (AAP)

Chlamydia: The AAP recommends annual screening for sexually active females 25 years old or younger. They also recommend annual urethral and rectal chlamydia screenings for sexually active MSM, but more frequent screening (every 3-6 months) for those who are in a higher risk category, such as multiple partners, sex-for-drugs, and so on. Anyone who has been exposed to chlamydia in the past 60 days should also be tested. "Consider screening sexually active males annually in settings with high prevalence rates, such as jails or juvenile corrections facilities, national job training programs, STD clinics, high school clinics, and adolescent clinics for patients who have a history of multiple partners." Anyone who has tested positive for chlamydia should be retested three months after receiving treatment.

Gonorrhea: Similar to chlamydia, the AAP recommends annual screening for sexually active females under the age of 25. "Routinely screen sexually active adolescent and young adults MSM for pharyngeal, rectal, and urethral gonorrhea infection annually if engaging in receptive oral or anal intercourse or insertive intercourse, respectively." Again, like chlamydial infections, those participating in higher risk activities should be tested every 3-6 months. Anyone who has been exposed to gonorrhea in the past 60 days should also be tested. Finally, the screening recommendations for other males are similar to the recommendations concerning chlamydial infections. Anyone who has tested positive for gonorrhea should be retested three months after receiving treatment.

Syphilis: "The routine screening of nonpregnant, heterosexual adolescents is not recommended. However, screening is recommended for all sexually active adolescent and young adults MSM annually or every 3 to 6 months if high risk and can be considered for youth whose behaviors put them at higher risk" (Murray et al., 2014).

National Institute for Health and Care Excellence (NICE)

NICE released their guidelines concerning cancer of the upper aerodigestive tract in 2016 (with updates in 2018 online). Recommendation 1.6.1: "Test all squamous cell carcinomas of the oropharynx using p16 immunohistochemistry. Regard the p16 test result as positive only if there is strong nuclear and cytoplasmic staining in more than 70% of tumour cells." In Recommendation 1.6.2: "Consider high-risk

HPV DNA or RNA in-situ hybridisation in all p16-positive cancers of the oropharynx to confirm HPV status." In explaining their recommendations, NICE states, "HPV testing is currently recommended in cancer of the oropharynx because it has significant prognostic implication" (NCCC, 2018).

Canadian Paediatric Society (CPS)

The 2018 update to the CPS practice point titled "Congenital syphilis: No longer just of historical interest" included the following:

"Syphilis serology should routinely be performed at the first prenatal visit, followed by appropriate maternal counselling and therapy, if reactive. Rescreening should occur at 28 to 32 weeks' gestation and at delivery in high-risk women, including women who originate from a country with a high prevalence of syphilis. Routine rescreening should also be considered in areas experiencing outbreaks of heterosexual syphilis. If syphilis serology was not performed during pregnancy, newborns should not be discharged from hospital until maternal serology has been drawn and follow-up of results has been arranged. If the cause is not known for a hydropic or stillbirth newborn, the mother should be screened for syphilis postpartum (Robinson & Canadian Paediatric Society, 2018)."

The CPS practice point sexually transmitted infections in adolescents: Maximizing opportunities for optimal care (Allen et al., 2019) included the following table concerning what screening tests should be used for each condition. These guidelines were updated in 2019, and reaffirmed in 2020 (Allen et al., 2019).

Table 1: What screening tests should be used use to detect sexually transmitted infections?

What screening tests should be used use to detect sexually transmitted infections?		
Infection	**Screening tests/samples**	**Follow-up testing**
Chlamydia	NAAT is the most sensitive and specific test. Can be performed on urine, urethral swabs, vaginal or cervical swabs* A culture of cervical or urethral specimen is the test of choice for medico-legal cases (eg., sexual assault). Confirmation by NAAT using a different set of primers or DNA sequencing may be used. For pharyngeal and rectal specimens, NAAT may be considered; discuss with testing laboratory	Test-of-cure 3 to 4 weeks after treatment: – Compliance is uncertain – Second-line or alternative treatment was used – Re-exposure risk is high – An adolescent is pregnant
Syphilis	Serology remains the usual diagnostic test unless the patient has lesions compatible with syphilis	Follow-up testing depends on the nature of infection, as follows:

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	Treponemal-specific screening assays (e.g., EIA) are more sensitive than non-treponemal tests, though testing algorithms vary across jurisdictions If treponemal-specific assay is positive, a second treponemal test is usually required	Primary, secondary, early latent infection: Repeat serology at 1, 3, 6, and 12 months after treatment Late latent infection: Repeat serology 12 and 24 months after treatment Neurosyphilis: Repeat 6, 12, and 24 months after treatment
Gonorrhea	**NAAT can be used to detect gonorrhea from urine, and urethral, vaginal and cervical swabs in symptomatic and asymptomatic individuals*** **Culture allows for antimicrobial susceptibility testing and should be performed if a patient does not promptly respond to therapy** **Cultures should be submitted for asymptomatic or symptomatic MSM, who have an increased incidence of antibiotic resistance** **For rectal and pharyngeal testing, discuss preferred specimens with the testing laboratory** **Culture is preferred for pharyngeal and rectal specimens** **For medico-legal purposes, a positive result obtained from NAATs should be confirmed using culture or a different set of primers, or by DNA sequencing techniques**	**Test-of-cure (culture 3 to 7 days post-treatment or NAAT 2 to 3 weeks later) if:** **– Second-line or alternative treatment was used** **– Antimicrobial resistance is a concern** **– Compliance is uncertain** **– Re-exposure risk is high** **– An adolescent is pregnant** **– Previous treatment failure** **– Pharyngeal or rectal infection** **– Infection is disseminated** **– Signs, symptoms persist post-treatment**

**Discuss specimen selection to ensure that the NAAT is validated for the specimen to be collected and the patient being tested. For example, NAAT testing has not been validated for children ≤12 years of age and for medico-legal specimens.*

British Association for Sexual Health and HIV (BASHH)

UK National Guideline for the Management of Lymphogranuloma Venereum (White et al., 2013): "Commercial molecular diagnostic techniques to detect *C. trachomatis* remain the primary test of choice, with referral of *C. trachomatis*-positive specimens for molecular tests to confirm the presence of LGV-associated DNA." Testing should be performed on anyone exhibiting symptoms of an LGV infection, including hemorrhagic proctitis, primary lesions, suspected LGV-associated pharyngitis, secondary lesions, buboes, lymphadenitis, and/or lymphadenopathy. Main diagnostic techniques include using either NAATs, "culture on cycloheximide-treated McCoy cells of material from suspected LGV lesions," or serology testing. "Serology cannot necessarily distinguish past from current LGV infection, which might prove restrictive given the high number of recurrent LGV infections now seen in MSM."

UK National Guideline for the Management of Anogenital Herpes (Patel et al., 2015): The clinical signs and symptoms of an HSV infection can include "painful ulceration, dysuria, vaginal or urethral discharge" as well as systemic symptoms of fever and myalgia. Other signs can include bilateral lymphadenitis—although, alternating sides can occur in subsequent episodes—and proctitis. With a Grade C recommendation, "The confirmation and typing of the infection and its type, by direct detection of HSV in genital lesions, are essential for diagnosis, prognosis, counselling, and management." BASHH gives an "A" recommendation of directly testing swabs from either anogenital lesions or the rectal mucosa in suspected proctitis. They recommend with a "B" rating that virus typing be performed to differentiate HSV-1 from HSV-2 in newly diagnosed cases of genital herpes. NAATs are the preferred testing method (grade "A" recommendation) since HSV culture tests can miss around 30% of PCR-positive samples.

UK National Guideline for the Management of Infection with Chlamydia Trachomatis (updated 2018) (Nwokolo et al., 2016): "Testing for genital and extra-genital chlamydia should be performed using NAATs (Grade B)." MSM who test positive for both HIV and chlamydia should be tested for LGV even if asymptomatic for the latter (Grade B). They give a Grade B recommendation for LGV testing in patients presenting with proctitis and a Grade C recommendation for treating both sexes presenting with proctitis the same.

The guidelines were updated in 2018, but NAAT testing is still considered the current standard of care for all chlamydia cases by the BASHH; "Although no test is 100% sensitive or specific, NAATs are known to be more sensitive and specific than EIAs" (BASHH, 2018).

UK National Guidelines on the Management of Syphilis (updated 2017, 2019) (Kingston et al., 2016): They recommend (2A) "where appropriate expertise and equipment are available, perform dark ground microscopy on possible chancres" and (1A) that "*T. pallidum* testing by PCR is appropriate on lesions where the organism may be expected to be located." Within the section on serology, they recommend (1B) that "An EIA/CLIA, preferably detecting both IgM and IgG is the screening test of choice"; "positive screening tests should be confirmed with a different treponemal test (not the FTA-abs) and a second specimen for confirmatory testing obtained" (1B); "a quantitative RPR or VDRL should be performed when screening tests are positive" (1A); and (1B) repeat testing for syphilis at 6 and 12 weeks if an isolated episode and "at two weeks after possible chancres that are dark-ground and/or PCR negative are

observed." These guidelines were updated in 2017 and 2019, but diagnostic testing methods were not changed.

Infectious Diseases Working Party of the German Society for Hematology and Medical Oncology (AGIHO/DGHO) and the German Working Group for Blood and Marrow Transplantation (DAG-KBT)

In 2016, the AGIHO/DGHO and the DAG-KBT released the "Infectious diseases in allogeneic haematopoietic stem cell transplantation: prevention and prophylaxis strategy guidelines 2016". In this guideline, they note that "comprehensive pre-transplant assessment of the allogeneic haematopoietic stem cell transplantation (allo-HCT) recipient for infectious complications is a valuable tool to identify patients at increased risk for distinct infectious diseases. All candidates for allo-HCT should undergo a test for IgG antibodies specific for syphilis infection. Serologic testing for syphilis is recommended. Frequently TPHA/TPPA or VDRL are utilized. Important are the combinations of nontreponemal (e.g. VDRL) and treponemal tests. If a nontreponemal test is positive, confirmation of infection with treponemal test (e.g. TPPA or TP-EIA) should be performed"(Ullmann, 2016).

Cumulative Guideline Table

Year & Society	Condition	Microorganism	Recommendation
2022 NCCN	Anal Carcinoma	HPV	HPV linked to anal cancers and HPV positivity linked to positive OS
2021 NCCN	Cervical Cancer	HPV	Overwhelming evidence of link between HPV and cervical cancer; chronic HPV infection status used in aiding treatment/surgical options
2022 NCCN	Head and Neck Cancers/ Oropharyngeal Cancer	HPV	Requires HPV p16 testing by IHC; HPV status is imperative in determining therapy
2021 NCCN	Occult Primary Cancers (Squamous Cell Carcinoma)	HPV	If clinical presentation in the head and neck nodes is noted, check p16 IHC and ISH results
2022 NCCN	Penile Cancer	HPV	HPV linked to penile cancer; HPV status of lesions important for determining therapy

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Year & Society	Condition	Microorganism	Recommendation
2021 NCCN	Vulvar Cancer (Squamous Cell Carcinoma)	HPV	HPV linked to vulvar cancer, especially HSIL; recommends HPV testing for "appropriate patients"
2021 USPSTF	NA	Chlamydia, Gonorrhea	Testing in sexually active women age 24 or younger and older women of at-risk populations; insufficient evidence concerning routinely screening in general population of males
2014 USPSTF	Oropharyngeal Cancer	HPV	Insufficient evidence to assess testing for HPV in cases of asymptomatic oropharyngeal cancer
2016 USPSTF	Asymptomatic Genital Herpes	HSV-2	Do not recommend testing asymptomatic patients for HSV-2
2016 USPSTF	NA	Syphilis	Grade A recommendation for screening asymptomatic patients of HIGH RISK categories but they do NOT recommend screening in asymptomatic patients not in high risk categories; recommend screening HIV-positive men and MSM every three months
2021 CDC	Genital, Anal, or Perianal Ulcers	Syphilis, HSV	Recommends syphilis serology, darkfield exam, or PCR testing if possible; culture or PCR for genital herpes; serologic testing for type-specific HSV antibody
2021 CDC	NA	Syphilis	Darkfield examination of exudate can be used for early diagnosis; presumptive diagnosis requires use of two tests—both a treponemal test and a non-treponemal test; any signs of CNS infection require additional testing

Diagnostic Testing of Common Sexually Transmitted Infections, continued

Year & Society	Condition	Microorganism	Recommendation
2021 CDC	NA	Chlamydia	Testing of women under age of 25 as well as older women and men if they fall in a high-risk category; do NOT recommend testing of asymptomatic men and older women
2021 CDC	NA	Gonorrhea	Testing of women under age of 25 as well as older women and men if they fall in a high-risk category; do NOT recommend testing of asymptomatic men and older women; men showing signs of urethral gonococcal infection should be tested
2021 CDC	NA	HPV	Recommends against using oncogenic HPV testing for asymptomatic men, women aged 25 and over, or for general STI testing. There is no approved test for HPV in men, and routine testing is not recommended for anal, penile, or throat cancers in men.
2021 CDC	Anogenital Warts	HPV	"HPV testing is not recommended for anogenital wart diagnosis, because test results are not confirmatory and do not guide genital wart management."

Diagnostic Testing of Common Sexually Transmitted Infections, continued

Year & Society	Condition	Microorganism	Recommendation
2021 CDC	Cervical Screening	HPV	For women aged 30 or older, HPV testing can be part of cervical screening. For women ages 30-65, if co-testing Pap test and HR-HPV, then frequency is every 5 years…if only doing a Pap test, the frequency is every 3 years HPV tests to screen for cervical cancer are recommended for women 30 years and older. They are not recommended to screen, men, adolescents, or women under the age of 30.
2019 IUSTI	Anogenital Warts	HPV	Do not recommend HPV testing for symptomatic anogenital warts since it adds no information for clinical use.
2020 IUSTI	NA	Gonorrhea	Culture testing is only method to determine antimicrobial susceptibility, but NAAT testing is more sensitive. Includes list of symptoms for testing.
2019 IUSTI	Lymphogranuloma venereum	Chlamydia	To diagnose LGV, a sample tested *C. trachomatis* positive with a commercial nucleic acid amplification test (NAAT) platform should be confirmed with an LGV discriminatory NAAT. For sensitive and specific LGV detection, laboratories are recommended to use a two-step procedure.
2014, 2020 IUSTI	NA	Syphilis	Like the CDC, they recommend a two-test method for diagnosing syphilis (one non-treponema test and one treponema test) if any initial screening test is positive

Diagnostic Testing of Common Sexually Transmitted Infections, continued

Year & Society	Condition	Microorganism	Recommendation
2015 IUSTI (published in 2016)	NA	Chlamydia	Recommends using an NAAT for chlamydia testing and lists signs/symptoms that require testing
2017 IUSTI	Genital herpes	HSV	Typically, does not recommend testing in asymptomatic patients; HSV DNA detection now replaces culture as gold standard
2014 MTC	NA	Chlamydia	Do not recommend pharyngeal screenings. Do recommend NAAT of at-risk groups with a 3-month follow-up test for patients who tested positive
2014 MTC	NA	Gonorrhea	Do recommend annual NAAT of at-risk groups with a 3-month follow-up test for patients who tested positive; more frequent testing in certain MSM populations
2014 MTC	NA	Syphilis	Do recommend annual testing of at-risk groups with 3-6 month testing of certain populations (commercial sex workers, inmates of correctional facilities, persons who exchange sex for drugs, and so on)
2017 Canadian Guidelines on STIs	NA	Chlamydia, Syphilis, Gonorrhea, HSV, and HPV	NAATs are more specific and sensitive than culture testing when available. For gonorrheal infections, only culture can test for antimicrobial susceptibility in gonorrhea.

Diagnostic Testing of Common Sexually Transmitted Infections, continued

Year & Society	Condition	Microorganism	Recommendation
2014 AAP	Adolescents & young adults	Chlamydia, Gonorrhea	All sexually active young women (under the age of 25) and MSM should have annual screenings. For those at higher risk, they should be screened every 3-6 months. Anyone who tests positive should be retested 3 months after receiving treatment.
2014 AAP	Adolescents & young adults	Syphilis	Do NOT recommend routine screening except for sexually active young MSM.
2016 NICE	Oropharyngeal Cancers	HPV	Test all carcinomas of the oropharynx using p16 IHC; consider using high-risk HPV DNA/RNA in situ hybridization in all p16-positive cancers
2018 CPS	Pregnant women	Syphilis	Testing at first prenatal visit as well as 28-32 weeks; if not tested during pregnancy, child does not leave the hospital without being tested
2020 CPS	Adolescents/young adults	Chlamydia, Syphilis, Gonorrhea	See detailed testing and frequency in table within the guidelines above
2015 BASHH (published in 2016)	NA	Syphilis	Dark-field microscopy or PCR tests can be performed. For serology, EIA/CLIA is the screening test of choice (preferably where both IgM and IgG are detected). Positive tests must be followed by a quantitative RPR or VDRL.
2013 BASHH	Suspected LGV	Chlamydia	Testing should use either NAAT, culture testing, or serology; however, the latter cannot distinguish current from past infections.

Diagnostic Testing of Common Sexually Transmitted Infections, continued

Year & Society	Condition	Microorganism	Recommendation
2014 BASHH (published in 2015)	Anogenital herpes	HSV	NAAT is preferred over other forms of testing ("A" grade). Differentiation of virus type should be determined on new cases of genital herpes ("B" grade).
2015, 2018 BASHH	NA	Chlamydia	Test for chlamydia using NAATs. Both sexes presenting with proctitis should be treated the same with respect to LGV testing. HIV-positive men with chlamydia should also be tested for LGV, even if asymptomatic.
Abbreviations: CLIA = chemiluminescent assay; EIA = enzyme immunoassay; GC = gonococcal; HPV = human papillomavirus; HR-HPV = high risk or oncogenic HPV testing; HSIL = high-grade squamous intraepithelial lesions; HSV = herpes simplex virus; IHC = immunohistochemistry; LGV = lymphogranuloma venereum; MSM = men having sex with men; NA = not applicable; NAAT = nucleic acid amplification testing; OS = overall survival; RPR = rapid plasma reagin test; VDRL = Venereal Diseases Research Laboratory carbon antigen test			

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

The FDA has approved many tests for HSV, chlamydia, gonorrhea, and syphilis. Some of these tests are discussed in the "Proprietary Testing" section of this policy. In addition to these tests, 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
82565	Creatinine; blood
82575	Creatinine; clearance
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
86592	Syphilis test, non-treponemal antibody; qualitative (e.g., VDRL, RPR, ART)
86593	Syphilis test, non-treponemal antibody; quantitative
86631	Antibody; Chlamydia
86701	Antibody; HIV-1
86702	Antibody; HIV-2
86703	Antibody; HIV-1 and HIV-2, single result
86704	Hepatitis B core antibody (HBcAb); total
86705	Hepatitis B core antibody (HBcAb); IgM antibody
86706	Hepatitis B surface antibody (HBsAb)
86632	Antibody; Chlamydia, IgM
86694	Antibody; herpes simplex, non-specific type test
86695	Antibody; herpes simplex, type 1
86696	Antibody; herpes simplex, type 2
86780	Antibody; Treponema pallidum
86803	Hepatitis C antibody
86804	Hepatitis C antibody; confirmatory test (eg, immunoblot)
87081	Culture, presumptive, pathogenic organisms, screening only;
87110	Culture, chlamydia, any source

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87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (e.g., antibiotic gradient strip)
87340	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; hepatitis B surface antigen (HBsAg)
87490	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, direct probe technique
87491	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, amplified probe technique
87492	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, quantification
87528	Infectious agent detection by nucleic acid (DNA or RNA); Herpes simplex virus, direct probe technique
87529	Infectious agent detection by nucleic acid (DNA or RNA); Herpes simplex virus, amplified probe technique
87530	Infectious agent detection by nucleic acid (DNA or RNA); Herpes simplex virus, quantification
87590	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, direct probe technique
87563	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma genitalium, amplified probe technique
87591	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, amplified probe technique
87592	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, quantification
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

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87660	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique
87661	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, amplified probe technique
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87808	Infectious agent antigen detection by immunoassay with direct optical observation; Trichomonas vaginalis
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
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
G0472	Hepatitis C antibody screening, for individual at high risk and other covered indication(s)
G0475	Hiv antigen/antibody, combination assay, screening
G0499	Hepatitis b screening in non-pregnant, high risk individual includes hepatitis b surface antigen (HBSAG) followed by a neutralizing confirmatory test for initially reactive results, and antibodies to HBSAG (anti-HBs) and Hepatitis B core antigen (anti-HBc)
S3645	HIV-1 antibody testing of oral mucosal transudate

0353U	Infectious agent detection by nucleic acid (DNA), Chlamydia trachomatis and Neisseria gonorrhoeae, multiplex amplified probe technique, urine, vaginal, pharyngeal, or rectal, each pathogen reported as detected or not detected
0354U	Human papilloma virus (HPV), high-risk types (ie, 16, 18, 31, 33, 45, 52 and 58) qualitative mRNA expression of E6/E7 by quantitative polymerase chain reaction (qPCR)
0402U	Infectious agent (sexually transmitted infection), Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium, multiplex amplified probe technique, vaginal, endocervical, or male urine, each pathogen reported as detected or not detected Proprietary test: Abbott Alinity™ m STI Assay Lab/Manufacturer: Abbott Molecular, 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
February 13, 2023	Added new coverage criteria #10 and removed previous coverage criteria #16 and #17; modified wording throughout remaining criteria. Also, added CPT codes **0353U** (covered with Select Health) and **0354U** (not covered with Select Health).
October 3, 2023	The following changes were implemented: Addition of coverage criteria #17 and #18: "17) NAATs or PCR-based testing for T. vaginalis **MEETS COVERAGE CRITERIA** in the following situations: a) Symptomatic individuals (see Note 7). b) Asymptomatic individuals belonging to a high-risk group: i) Concurrent STI or history of STIs. ii) Individuals in high prevalence settings, such as STI clinics. iii) Individuals who exchange sex for payment. 18) Rapid identification of trichomoniasis by enzyme immunoassay **DOES NOT MEET COVERAGE CRITERIA**." Coverage criteria #2, #5, and #9, which address screening for syphilis, chlamydia, and gonorrhea, respectively, in asymptomatic individuals without high-risk factors, new sub-criteria: "c) For sexually active individuals less than 18 years of age (annually)." was added to each.
January 8, 2024	The following changes were implemented: added new coverage criteria #1d: "Treponemal Ig testing and nontreponemal testing (once prior to transplant) as a part of a pre-transplant assessment in both donors and recipients of an allogeneic hematopoietic stem cell transplantation (allo-HCT)."; moved coverage

Diagnostic Testing of Influenza

Policy #: AHS – G2119	**Prior Policy Name & Number (as applicable):** • G2119 – Rapid Flu Tests in the Outpatient Setting • Also included influenza coverage from prior M2097 – Identification of Microorganisms Using Nucleic Acid Probes
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

Influenza is an acute respiratory illness caused by influenza A or B viruses resulting in upper and lower respiratory tract infection, fever, malaise, headache, and weakness. It mainly occurs in outbreaks and epidemics during the winter season, and is associated with increased morbidity and mortality in certain high-risk populations (Dolin, 2020).

Rapid influenza diagnostic tests (RIDTs) refer to clinical laboratory improvement amendments (CLIA) waived immunoassays that can detect influenza viruses during the outpatient visit, giving results in a clinically relevant time period to inform treatment decisions (CDC, 2017). Besides RIDTs, influenza can be detected using polymerase chain reaction (PCR)-based assays as well as culture testing; however, the former is not often used in initial clinical management due to time constraints. Serologic testing is not used in outpatient settings for diagnosis (Dolin, 2019b).

II. Related Policies

Policy Number	Policy Title
AHS-G2149	Pathogen Panel Testing
AHS-G2174	Coronavirus Testing in the Outpatient Setting

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 diagnosis in patients who present in the outpatient setting with signs and symptoms consistent with influenza disease (See Note 1 below) when influenza activity has been documented in the community or geographic area, ONE, but NOT BOTH, of the following **MEETS COVERAGE CRITERIA**:

   a) One single rapid flu test- includes either a point-of-contact rapid nucleic acid amplification test (NAAT) or a rapid antigen test; or
   b) One single traditional NAAT.

2) Viral culture testing for influenza in an outpatient setting **DOES NOT MEET COVERAGE CRITERIA**.

3) In asymptomatic patients, outpatient influenza testing, including rapid antigen flu tests, rapid NAAT or RT-PCR tests, traditional RT-PCR tests, and viral culture testing **DOES NOT MEET COVERAGE CRITERIA**.

4) Serology testing for influenza **DOES NOT MEET COVERAGE CRITERIA** under any circumstance.

Note 1: Typical Influenza Signs and Symptoms (CDC, 2018)

- Fever: A 100.4°F or higher temperature or feeling feverish/chills AND one or more:
  - Cough
  - Sore throat
  - Headaches and/or body aches
  - Difficulty breathing or shortness of breath
  - Fatigue
  - Runny or stuffy nose

IV. Scientific Background

The influenza virus causes seasonal epidemics that result in severe illnesses and death every year. Influenza characteristically begins with the abrupt onset of fever, headache, myalgia, and malaise (Dolin, 1976; Kilbourne & Loge, 1950; Loeb et al., 2012; Nicholson, 1992), accompanied by manifestations of respiratory tract illness, such as nonproductive cough, sore throat, and nasal discharge (Dolin, 2022b).

High titers of influenza virus are often present in respiratory secretions of infected persons. Influenza is transmitted primarily via respiratory droplets produced from sneezing and coughing (Brankston et al., 2007; Dolin, 2022b; Mubareka et al., 2009) which requires close contact with an infected individual. The typical incubation period for influenza is one to four days (average two days) (CDC, 2017; Cox & Subbarao, 1999). The serial interval among household contacts is three to four days (Cowling et al., 2010). When initiated promptly (within the first 24 to 30 hours), antiviral therapy can shorten the duration of influenza symptoms by approximately one-half to three days (Cooper et al., 2003; Dobson, et al., 2015; Hayden et al., 1997; Heneghan et al., 2014; Jefferson et al., 2014; Nicholson et al., 2000; Zachary, 2022).

In certain circumstances, the diagnosis of influenza can be made clinically, such as during an outbreak. At other times, it is important to establish the diagnosis using laboratory testing. Viral diagnostic test options include rapid antigen tests, immunofluorescence assays, and reverse-transcriptase polymerase chain reaction (RT-PCR)-based testing (CDC, 2017). Among these, RT-PCR is the most sensitive and specific (Dolin, 2022a). Rapid influenza antigen tests are immunoassays that can identify influenza A and B viral nucleoprotein antigens in respiratory specimens (CDC, 2017) which yield qualitative results in approximately 15 minutes or less. However, they have much lower sensitivity (CDC, 2017; Harper et al., 2009; Hurt et al., 2007; Ikenaga et al., 2008). A recent meta-analysis found that the sensitivity of these immunoassays was 62.3 percent and the specificity was 98.2 percent (Chartrand, Leeflang, Minion, Brewer, & Pai, 2012). Furthermore, detectable viral shedding in respiratory secretions peaks at 24 to 48 hours of illness and then rapidly declines (Dolin, 2022a).

A decision analysis by Sintchenko et al. concluded that treatment based on rapid diagnostic testing results was appropriate first over empirical antiviral treatment, except during influenza epidemics. When the probability of a case being due to influenza reached 42 percent, the two strategies were equivalent. Further, a separate meta-analysis found that rapid diagnostic testing did not add to the overall cost-effectiveness of treatment if the probability of influenza was greater than 25 to 30 percent (Call et al., 2005; Dolin, 2022a).

Analytical Validity

Viral culture is a gold standard for influenza diagnosis, but it is very time-consuming with an average 7-day turnaround time; on the other hand, real-time RT-PCR and shell vial (SV) testing require only an average of 4 hours and 48 hours, respectively. A study by Lopez Roa et al. (2011) compared real-time RT-PCR and SV testing against conventional cell culture to detect pandemic influenza A H1N1. The sensitivity of real-time RT-PCR as compared to viral culture testing was 96.5%, and SV had a sensitivity of

73.3% and 65.1%, depending on the use of either A549 cells or Madin-Darby Canine Kidney (MDCK) cells, respectively. The authors conclude, "Real-time RT-PCR displayed high sensitivity and specificity for the detection of influenza A H1N1 in adult patients when compared with conventional techniques" (Lopez Roa et al., 2011).

Clinical Validity and Utility

In 2017, Yoon et al. (2017) investigated the use of saliva specimens for detecting influenza A and B using RIDTs. Both saliva and nasopharyngeal swab (NPS) samples were analyzed from 385 patients; each sample was assayed using four different RIDTs—the Sofia Influenza A+B Fluorescence Immunoassay, ichroma TRIAS Influenza A+B, SD Bioline Influenza Ag, and BinaxNOW Influenza A/B antigen kit—as well as real-time RT-PCR. Using real-time RT-PCR as a standard, 31.2% of the patients tested positive for influenza A and 7.5% for influenza B. All four RIDTS had "slightly higher" diagnostic sensitivity in NPS samples than saliva samples; however, both Sofia and ichroma "were significantly superior to those of the other conventional influenza RIDTs with both types of sample" (Yoon et al., 2017). The authors note that the sensitivity of diagnosis improves if both saliva and NPS testing is performed (from 10% to 13% and from 10.3% to 17.2% for A and B, respectively). The researchers conclude, "This study demonstrates that saliva is a useful specimen for influenza detection, and that the combination of saliva and NPS could improve the sensitivities of influenza RIDTs" (Yoon et al., 2017).

Ryu et al. (2016) investigated the efficacy of using instrument-based digital readout systems with RIDTs. In their 2016 paper, the authors included 314 NPS samples from patients with suspected influenza and tested each sample with the Sofia Influenza A+B Fluorescence Immunoassay and BD Veritor System Flu A+B, which use instrument-based digital readout systems, as well as the SD Bioline assay (a traditional immunochromatographic assay) and PCR, the standard. Relative to the RT-PCR standard, for influenza A, the sensitivities for the Sofia, BD Veritor, and SD Bioline assays were 74.2%, 73.0%, and 53.9%, respectively; likewise, for influenza B, the sensitivities, respectively, were 82.5%, 72.8%, and 71.0%. All RIDTS show 100% specificities for both subtypes A and B. The authors conclude, "Digital-based readout systems for the detection of the influenza virus can be applied for more sensitive diagnosis in clinical settings than conventional [RIDTs]" (Ryu et al., 2016). Similar research was performed in 2018 on NPS using RIDTs with digital readout systems—Sofia and Veritor as before along with BUDDI—as compared to standard RT-PCR and the SD Bioline immunochromatographic assay (n=218). The four RIDTs were also tested with diluted solutions from the National Institute for Biological Standards and Control (NIBSC) to probe lower detection limits for each testing method. Again, the digital-based assays exhibited higher sensitivity for influenza. "Sofia showed the highest sensitivity for influenza A and B detection. BUDDI and Veritor showed higher detection sensitivity than a conventional RIDT for influenza A detection. Further study is needed to compare the test performance of RIDTs according to specific, prevalent influenza subtypes" (Ryu et al., 2018).

Another study compared the Alere iNAT, a rapid isothermal nucleic acid amplification assay, to the Sofia Influenza A+B and the BinaxNOW Influenza A&B immunochromatographic (ICT) assay. Using RT-PCR as the standard for 202 NPS samples, the "Alere iNAT detected 75% of those positive by RT-PCR, versus 33.3% and 25.0% for Sofia and BinaxNOW, respectively. The specificity of Alere iNAT was 100% for influenza A and 99% for influenza B" (Hazelton et al., 2015). BinaxNOW also had a sensitivity of only 69%

for influenza as compared to RT-PCR in another study of 520 NPS from children under the age of 5 (Moesker et al., 2016).

Young et al. (2017) investigated the accuracy of using point-of-care (POC) nucleic acid amplification test (NAAT)-based assays on NPS as compared to the US Food and Drug Administration (FDA)-cleared in vitro PCR test, GenMark Dx Respiratory Viral Panel. Their study consisted of 87 NPS samples from adults. As compared to the RT-PCR gold standard, the cobas Liat Influenza A/B POC test had an overall sensitivity and specificity of 97.9% and 97.5%, respectively, whereas the Alere i Influenza A&B POC test's sensitivity was only 63.8% with a specificity of 97.5% (Young et al., 2017). Taken together, the authors conclude that "the cobas Influenza A/B assay demonstrated performance equivalent to laboratory-based PCR, and could replace rapid antigen tests" (Young et al., 2017). These results are corroborated by another study that measured the specificity of the cobas POC assay as 100% for influenza A/B with a sensitivity of 96% for influenza A and 100% for influenza B (Melchers et al., 2017). Further, a third study reported a 6.5% invalid rate (as defined by as a failure on a first-run assay) by the cobas POC assay; however, "the sensitivities and specificities for all assays [cobas, Xpert Xpress Flu/RSV, and Aries Flu A/B & RSV] were 96.0 to 100.0% and 99.3 to 100% for all three viruses [influenza A, influenza B, and respiratory syncytial virus]" (Ling et al., 2018).

Kanwar et al. (2020) compared three rapid, POC molecular assays for influenza A and B detection in children: the ID Now influenza A & B assay, the Cobas influenza A/B NAAT, and Xpert Xpress Flu. Each of the three aforementioned tests are CLIA-waived influenza assays. PCR was used to compare results from each. NPS Samples from 201 children were analyzed for this study. The researchers note that "The overall sensitivities for the ID Now assay, LIAT, and Xpert assay for Flu A virus detection (93.2%, 100%, and 100%, respectively) and Flu B virus detection (97.2%, 94.4%, and 91.7%, respectively) were comparable. The specificity for Flu A and B virus detection by all methods was >97%" (Kanwar et al., 2020).

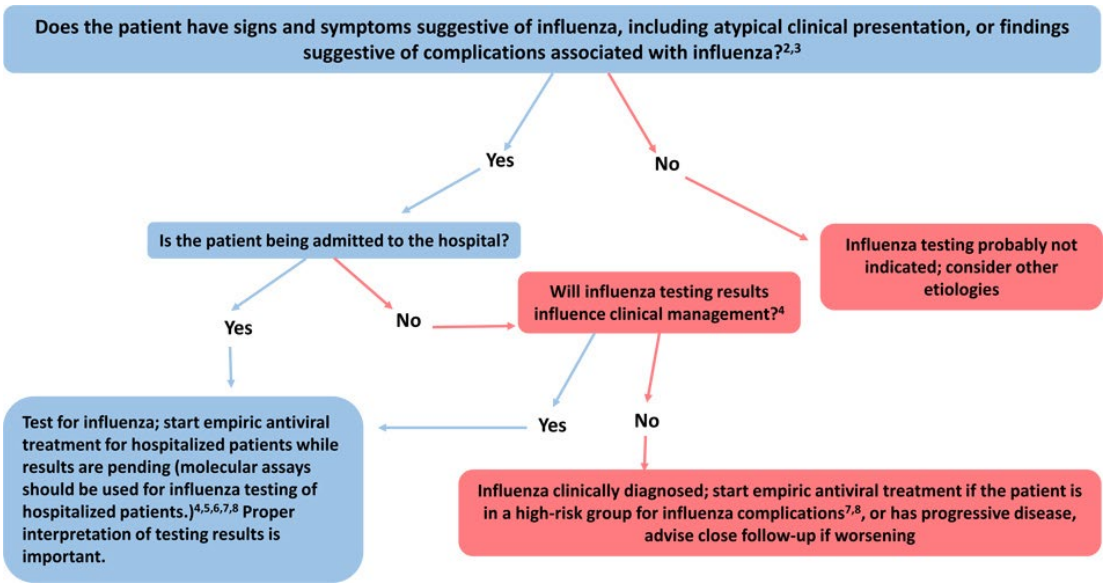
Sato et al. (2022) conducted a study comparing the results from rapid antigen detection (Quick Chaser Flu A, B), silver amplified immunochromatography (Quick Chaser Auto Flu A, B), and two separate NAATs (Xpert Xpress Flu/RSV and cobas Influenza A/B & RSV). The researchers also used a baseline RT-PCR assay as a reference for the study results. The sensitivities of the rapid antigen detection test and silver amplified immunochromatography test were 41.7% and 50.0% <6 hours after onset, but both were 100% in sensitivity at 24-48h after onset. Ultimately, the researchers concluded that the two NAATs had comparable analytical performances, whereas the rapid antigen detection and silver amplified immunochromatography tests had increased false negatives oftentimes when viral load is low in early infection.

V. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

The CDC gives two sets of guidelines concerning testing for influenza. If influenza is known to be circulating in the community, they give the algorithm displayed in the figure below (CDC, 2019a):

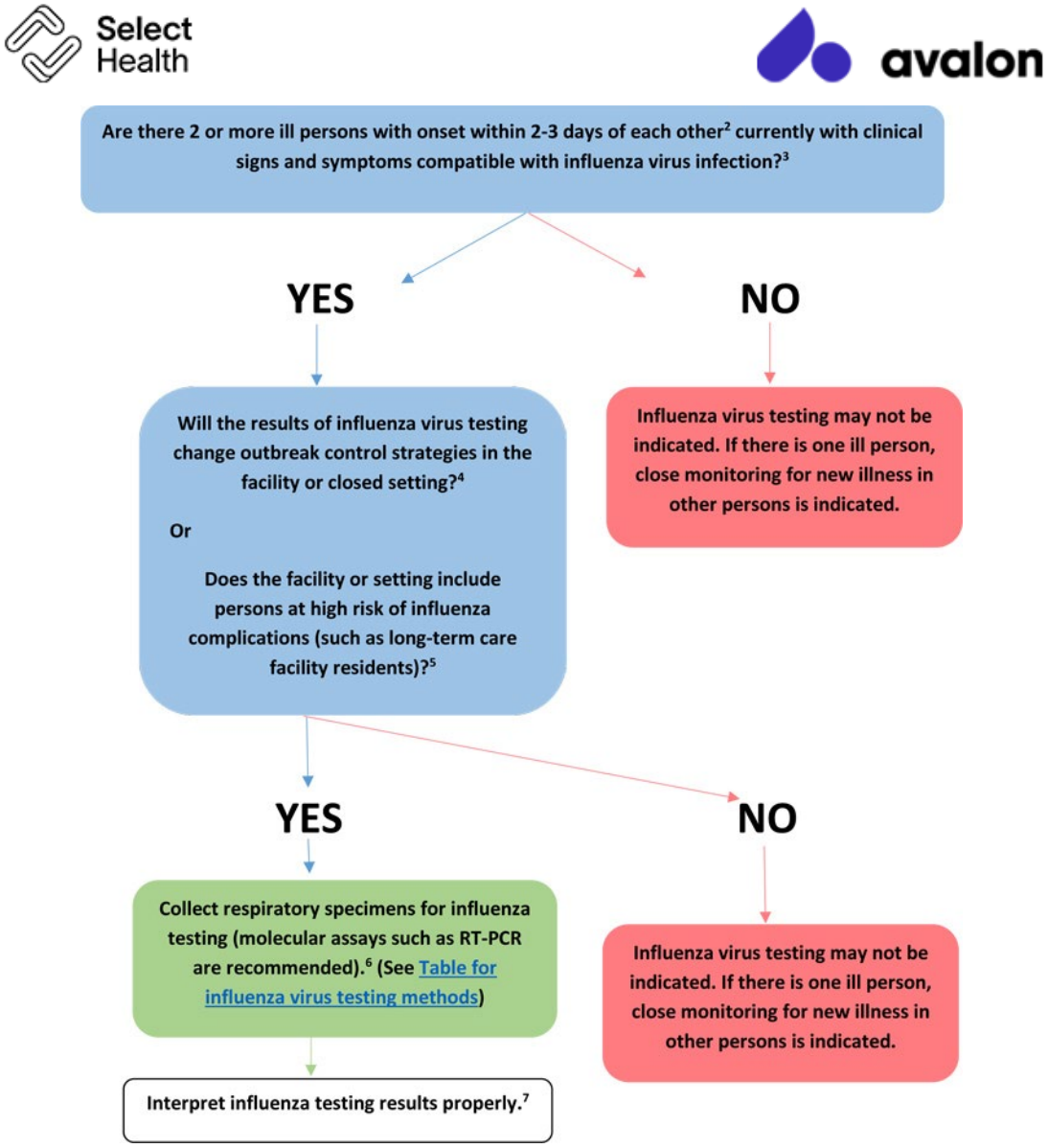
Diagnostic Testing of Influenza, continued



If the patient is asymptomatic for influenza, then they do not recommend testing. If the patient is symptomatic and is being admitted to the hospital, then they recommend testing; on the other hand, if a symptomatic patient is not being admitted to the hospital, they recommend testing if the results of the test will influence clinical management. Otherwise, if the test results are not going to influence the clinical management, then do not test but do administer empiric antiviral treatment for any patient in high-risk categories (CDC, 2020b). [For a list of typical signs and symptoms of influenza according to the CDC, please refer to Note 1 within the Coverage criteria section above (CDC, 2020a).]

For possible outbreaks in a closed setting or institution, the CDC issued the guideline algorithm in the figure below (CDC, 2019b):

Diagnostic Testing of Influenza, continued



If only one person is showing signs and symptoms of influenza, then testing is not recommended but he/she should be closely monitored. If multiple people are showing signs of influenza, then RT-PCR testing is recommended if the results would change control strategies or if there are persons at high risk of complications within the facility or closed setting (CDC, 2019). [For a list of signs and symptoms and a list of high-risk populations, please see Notes 1 and 2, respectively, in the Coverage criteria section below] (CDC, 2018).

The CDC notes the usefulness of RIDT influenza testing given the rapid testing time (less than 15 minutes on the average) and that some have been cleared for point-of-care use, but they note the limited sensitivity to detect influenza as compared to the reference standards for laboratory confirmation

testing, RT-PCR or viral culture. Disadvantages of RIDTs include high false negative results, especially during outbreaks, false positive results during times when influenza activity is low, and the lack of parity in RIDTs in detecting viral antigens. "Testing is not needed for all patients with signs and symptoms of influenza to make antiviral treatment decisions (See Figures 1-4). Once influenza activity has been documented in the community or geographic area, a clinical diagnosis of influenza can be made for outpatients with signs and symptoms consistent with suspected influenza, especially during periods of peak influenza activity in the community (CDC, 2017)."

The CDC notes the practicality of using RIDTs to detect possible influenza outbreaks, especially in closed settings. "RIDTs can be useful to identify influenza virus infection as a cause of respiratory outbreaks in any setting, but especially in institutions (i.e., nursing homes, chronic care facilities, and hospitals), cruise ships, summer camps, schools, etc. Positive RIDT results from one or more ill persons with suspected influenza can support decisions to promptly implement infection prevention and control measures for influenza outbreaks. However, negative RIDT results do not exclude influenza virus infection as a cause of a respiratory outbreak because of the limited sensitivity of these tests. Testing respiratory specimens from several persons with suspected influenza will increase the likelihood of detecting influenza virus infection if influenza virus is the cause of the outbreak, and use of molecular assays such as RT-PCR is recommended if the cause of the outbreak is not determined and influenza is suspected. Public health authorities should be notified promptly of any suspected institutional outbreak and respiratory specimens should be collected from ill persons (whether positive or negative by RIDT) and sent to a public health laboratory for more accurate influenza testing by molecular assays and viral culture." The CDC recommends using a molecular assay, such as RT-PCR, to test any hospitalized individual with suspected influenza rather than using an RIDT (CDC, 2017).

Infectious Diseases Society of America (IDSA)

The IDSA published an update to seasonal influenza in adults and children in 2018. The following three recommendations relating to outpatient influenza testing were published:

- "Clinicians should use rapid molecular assays (ie, nucleic acid amplification tests) over rapid influenza diagnostic tests (RIDTs) in outpatients to improve detection of influenza virus infection."
- "Clinicians should not use viral culture for initial or primary diagnosis of influenza because results will not be available in a timely manner to inform clinical management (A-III), but viral culture can be considered to confirm negative test results from RIDTs and immunofluorescence assays, such as during an institutional outbreak, and to provide isolates for further characterization."
- "Clinicians should not use serologic testing for diagnosis of influenza because results from a single serum specimen cannot be reliably interpreted, and collection of paired (acute/convalescent) sera 2–3 weeks apart are needed for serological testing (Uyeki et al., 2018)."

The 2018 IDSA guidelines for the diagnosis of infectious diseases by microbiology laboratories (Miller et al., 2018) under viral pneumonia respiratory infections, specifically including influenza, state: "Rapid antigen tests for respiratory virus detection lack sensitivity and depending upon the product, specificity.

Diagnostic Testing of Influenza, continued

A recent meta-analysis of rapid influenza antigen tests showed a pooled sensitivity of 62.3% and a pooled specificity of 98.2%. They should be considered as screening tests only. At a minimum, a negative result should be verified by another method… Several US Food and Drug Administration (FDA)-cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected (Miller et al., 2018)." Moreover, they state that the "IDSA/American Thoracic Society (ATS) practice guidelines (currently under revision) consider diagnostic testing as optional for the patient who is not hospitalized." For children, though, they do recommend testing for viral pathogens in both outpatient and inpatient settings. In the section on general influenza virus infection, again they recommend the use of rapid testing assays, noting the higher sensitivity of the NAAT-based methods over the rapid antigen detection assays. They also state: Serologic testing is not useful for the routine diagnosis of influenza due to high rates of vaccination and/or prior exposure (Miller et al., 2018)."

American Academy of Emergency Medicine (AAEM)

The AAEM approved a clinical practice paper on influenza in the emergency department: vaccination, diagnosis, and treatment. This document gives a "Level B" recommendation that states: "Testing for influenza should only be performed if the results will change clinical management. If a RAD [rapid antigen diagnostic] testing method is utilized, the provider should be aware of the limited sensitivity and the potential for false negatives. If clinical suspicion is moderate to high and RAD test is negative, one should consider sending a confirmatory RT-PCR or proceeding with empiric treatment for suspected influenza (Abraham et al., 2016)." This guideline has since been archived on the AAEM website.

Committee on Infectious Diseases, American Academy of Pediatrics (AAP), 32nd Edition (2021-2024, Red Book)

The Committee on Infectious Diseases released joint guidelines with the American Academy of Pediatrics. These joint guidelines recommend that "influenza testing should be performed when the results are anticipated to influence clinical management (eg, to inform the decision to initiate antiviral therapy or antibiotic agents, to pursue other diagnostic testing or to implement infection prevention and control measures)" (AAP, 2021).

Regarding types of testing, the AAP states that "The decision to test is related to the level local influenza activity, clinical suspicion for influenza, and the sensitivity and specificity of commercially available influenza tests… These include rapid molecular assays for influenza RNA or nucleic acid detection, reverse transcriptase-polymerase chain reaction (RT-PCR) single-plex or multiplex assays, real time or other RNA-based assays, immunofluorescence assays (direct [DFA] or indirect [IFA] fluorescent antibody staining) for antigen detection, rapid influenza diagnostic tests (RIDTs) based on antigen detection, rapid cell culture (shell vial culture), and viral tissue cell culture (conventional) for virus isolation. The optimal choice of influenza test depends on the clinical setting" (AAP, 2021).

National Institute of Health (NIH)

The NIH published a webpage on influenza diagnoses. This page notes that "Diagnostics that enable healthcare professionals to quickly distinguish one flu strain from another at the point of patient care

and to detect resistance to antiviral drugs would ensure that patients receive the most appropriate care" (NIH, 2017).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On 1/12/2017, the FDA released the following concerning the reclassification of influenza testing systems: "The Food and Drug Administration (FDA) is reclassifying antigen based rapid influenza virus antigen detection test systems intended to detect influenza virus directly from clinical specimens that are currently regulated as influenza virus serological reagents from class I into class II with special controls and into a new device classification regulation" (Kux, 2017). The effective date is 2/13/2017. This reclassification now requires new minimum standards and annual reactivity testing. "Consequently, many previously available RIDTs can no longer be purchased in the United States" (Azar & Landry, 2018).

A list of tests granted waived status under CLIA (Clinical Laboratory Improvement Amendments of 1988) according to CPT codes is maintained by the Centers for Medicare & Medicaid Services (CMS) website (CMS, 2018). As of 7/24/2018, 27 different influenza tests are listed with the 87804 CPT code for influenza immunoassay with direct optical observation.

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
86710	Antibody; influenza virus
87254	Virus isolation; centrifuge enhanced (shell vial) technique, includes identification with immunofluorescence stain, each virus
87275	Infectious agent antigen detection by immunofluorescent technique; influenza B virus
87276	Infectious agent antigen detection by immunofluorescent technique; influenza A virus
87400	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; Influenza, A or B, each
87501	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, includes reverse transcription, when performed, and amplified probe technique, each type or subtype

87502	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, first 2 types or sub-types
87503	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, each additional influenza virus type or sub-type beyond 2 (List separately in addition to code for primary procedure)
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
87804	Infectious agent antigen detection by immunoassay with direct optical observation; Influenza

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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Diagnostic Testing of Influenza, continued

Diagnostic Testing of Iron Homeostasis & Metabolism

Policy #: AHS – G2011	**Prior Policy Name & Number (as applicable):** AHS-G2011-Ferritin
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

Iron, an essential nutrient with a variety of biological uses, is tightly regulated *in vivo* to maintain homeostasis. Enterocytes absorb iron as Fe^{2+} either in its non-heme form via DMT1 (divalent metal-ion transporter-1) or in heme form presumably through receptor-mediated endocytosis. The enterocytes then release iron through ferroportin where transferrin binds it as biologically inactive Fe^{3+}. Saturated transferrin delivers iron to erythrocyte precursors in bone marrow where it is incorporated into hemoglobin during erythropoiesis. Transferrin may also salvage iron released by the reticuloendothelial system and macrophages (Knutson, 2017).

All cells require iron; consequently, saturated transferrin can also bind to its receptors (TfR1 or TfR2). The bound transferrin receptor (TfR) undergoes receptor-mediated endocytosis followed by export of divalent iron for cellular use (Byrne et al., 2013). Intracellularly, iron is stored within the central cavity of the protein ferritin, a large spherical protein that can store up to 4500 iron atoms per protein. Ferritin has ferroxidase activity required for iron uptake and storage. In conjunction with transferrin and TfR, ferritin is an acute phase reactant that responds to oxidative stress and inflammation (Camaschella, 2022). Moreover, TfR1 and TfR2, upon activation by transferrin, can initiate signaling cascades required for hepcidin expression (Roetto et al., 2018). Hepcidin, a small peptide hormone, acts as a modulator of serum iron concentrations by binding to ferroportin, the only iron exporter; ultimately, this results in the degradation of ferroportin and an intracellular accumulation of iron (Pietrangelo, 2015).

Please note that carbohydrate-deficient transferrin is out of scope for this policy.

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. Measurement of serum ferritin levels **MEETS COVERAGE CRITERIA** in any of the following situations:
    a. For the evaluation of an individual with abnormal hemoglobin and/or hematocrit levels.
    b. For the evaluation and monitoring of iron overload disorders.
    c. For individuals with symptoms of hemochromatosis (see Note 1).
    d. For individuals with first-degree relatives with confirmed hereditary hemochromatosis (HH)
    e. For the evaluation of individuals with liver disease.
    f. For the evaluation of hemophagocytic lymphohistiocytosis (HLH) and Still's Disease.
    g. In males with secondary hypogonadism.
    h. At a frequency of every 1 to 3 months:
        i) For the evaluation and monitoring of patients with chronic kidney disease who are receiving or being considered for receiving treatment for anemia.
        ii) For individuals on iron therapy.
2. Measurement of serum transferrin saturation (using serum iron and serum iron binding capacity measurements) **MEETS COVERAGE CRITERIA** in any of the following:
    a. For the evaluation of iron overload in individuals with symptoms of hemochromatosis (see Note 1).
    b. For the evaluation of iron overload in individuals with first-degree relatives (see Note 2) with confirmed hereditary hemochromatosis (HH).
    c. For the evaluation of iron deficiency anemia.
3. In asymptomatic patients, the use of ferritin or transferrin measurement, including transferrin saturation, as a screening test **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. Serum hepcidin testing, including immunoassays, **DOES NOT MEET COVERAGE CRITERIA**.
5. The use of GlycA testing to measure or monitor transferrin or other glycosylated proteins **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

NOTE 1: Symptoms of hemochromatosis, according to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health include the following (NIDDK, 2014):

- Joint pain
- Fatigue
- Unexplained weight loss
- Abnormal bronze or gray skin color
- Abdominal pain
- Loss of sex drive

NOTE 2: First-degree relatives include parents, full siblings, and children of the individual.

IV. Scientific Background

Iron is necessary for fundamental metabolic processes and acts as the central component in the catalytic sites of numerous essential enzymes and multiprotein complexes, such as mitochondrial respiratory chain complexes and oxygen binding proteins (Hentze et al., 2004; Zhang et al., 2014). Tight regulation of iron metabolism for maintaining adequate iron levels is achieved by the interaction of a number of iron metabolism-related proteins (Zhang et al., 2014) as well as the hemostatic modulation of iron absorption, utilization, and recycling (Hentze et al., 2010). This strict regulation is pertinent due to the potential toxicity of iron from its redox reactivity and the resultant generation of damaging free radicals (Finazzi & Arosio, 2014).

Several mechanisms in the body regulate the dietary absorption of iron and its concentration in other areas, such as plasma and extracellular milieu; this process is known as systemic iron homeostasis (Ganz, 2013). Iron homeostasis is a complex process where the small peptide hormone hepcidin plays a major role by binding the sole mammalian iron exporter, ferroportin. This leads to ferroportin degradation by lysosomes. Furthermore, hepcidin production is sensitive to extracellular iron concentrations by way of the human homeostatic iron regulator (HFE) protein and the transferrin receptors (TfRs). The HFE protein has been shown to interact with both TfR1 and TfR2, initiating the BMP-SMAD signaling pathway upon transferrin binding. This signaling cascade ultimately increases expression of the *HAMP* gene that encodes for hepcidin (Pietrangelo, 2015; Vujić, 2014).

Ferritins are a highly conserved family of proteins that detoxify and store excess iron as less reactive ferrihydrite (Hentze et al., 2004). This intracellular iron storage mechanism allows the cell to maintain and utilize spare iron based on changes in metabolic demand (Finazzi & Arosio, 2014). Mammalian

ferritins are heteropolymers comprised of tissue-specific combinations of 24 subunits. These subunits consist of two types: Ferritin L (FTL) and Ferritin H (FTH); a spherical structure is formed from these subunits, facilitating the dynamic storage of iron (Finazzi & Arosio, 2014; Liu & Theil, 2005). The levels and composition of ferritin are regulated by oxidative stress at the transcriptional level (Arosio & Levi, 2010; Bresgen & Eckl, 2015), and by iron responsive proteins (IRP) at the post-transcriptional level (Anderson et al., 2012). Several tissues express a mitochondria-specific ferritin protein that further protect these mitochondria from oxidative damage (Campanella et al., 2009; Paul et al., 2017).

Iron is released as needed from ferritin by ferritinophagy, the targeting of ferritin for degradation by lysosomes; this process requires cargo protein nuclear receptor coactivator 4 (NCOA4), as NCOA4-deficient cells cannot degrade ferritin correctly (Mancias et al., 2014). After release, the iron is transported back to the cytosol by divalent metal transporter 1 (DMT1) (La et al., 2018). This process allows the iron to become available as part of the labile iron pool (Cabantchik, 2014; Kruszewski, 2003).

Degradation of ferritin and resultant accumulation of lethal reactive oxygen species (ROS) has been recognized as a distinct iron-dependent type of regulated, non-apoptotic cell death known as ferroptosis (Hou et al., 2016; Xie et al., 2016). Dysregulated ferroptosis has been implicated in neurotoxicity, neurodegenerative diseases, acute renal failure, drug-induced hepatotoxicity, hepatic and heart ischemia/reperfusion injury, and T-cell immunity (Xie et al., 2016). Abnormal ferroptosis has also been recently found to play a role in drug treatment, particularly in decitabine treatment of myelodysplastic syndrome (MDS). The drug-induced ROS release decreases glutathione (GSH) and glutathione peroxidase 4 (GPX4), features characteristic of this unique cell-death process (Lv et al., 2020).

Ferritin can routinely be detected in serum (Alfrey, 1978) as a result of secretion from macrophages (Cohen et al., 2010) or release during cell death and lysis (Kell & Pretorius, 2014). Serum ferritin (SF) is primarily composed of L subunits, contains relatively little iron, and is partially glycosylated (Santambrogio et al., 1987; Wang et al., 2010). Causes of elevated SF levels include, but are not limited to, acute or chronic inflammation, chronic alcohol consumption, liver disease, renal failure, metabolic syndrome, or malignancy rather than iron overload (Koperdanova & Cullis, 2015). In healthy adults, levels of SF generally reflect overall iron storage (Costa Matos et al., 2013; Enko et al., 2015; Finch et al., 1986; Jacobs et al., 1972; Wang et al., 2010; Zanella et al., 1989). This closely correlates with the “gold standards” of measuring iron stores in bone marrow or liver biopsy (Peng & Uprichard, 2017).

Given that iron is an essential component for many metabolic processes, the immune system has developed mechanisms for iron sequestration as part of the inflammatory response in order to prevent invading pathogens and tumors from utilizing iron (Wang et al., 2010). Hence, increased levels of SF during the immune system-based acute phase response do not necessarily correlate with iron availability or stores, but rather are a general indicator of inflammation (Dignass et al., 2018). This becomes a critical issue when assessing iron deficiency (ID), as elevations in SF during inflammation can mask the presence of ID (Suchdev et al., 2017). However, this makes the assessment of iron status in the presence of inflammation more complex (Dignass et al., 2018; Knovich, Storey, Coffman, Torti, & Torti, 2009; Munoz, Gomez-Ramirez, et al., 2017). Additionally, the two subunits of ferritin (FTL and FTH) have been reported to differentially locate during periods of inflammation; this complicates the use of these subunits as an inflammatory diagnostic tool (Ahmad et al., 2013). In analyzing data from the Biomarkers Reflecting the Inflammation and Nutritional Determinants of Anemia (BRINDA) project,

Suchdev et al. (2017) identified that all their examined indicators of iron status (SF, serum TfR, total body iron) were affected by inflammation, and suggested utilizing C-reactive protein (CRP), a measure of acute inflammation, and α1-acid glycoprotein, a measure of chronic inflammation, in addition to iron indicators to better account for the full range and severity of inflammation.

Extremely elevated SF, in excess of five times the upper limit of normal (Evensen et al., 2007), can indicate adult-onset Still disease. Still disease is a systemic inflammatory disorder that typically affects young women and is characterized by fever, arthritis, and rash (Knovich et al., 2009; Zandman-Goddard & Shoenfeld, 2007). More extremely elevated SF (above 10,000 ug/L), especially in the context of autoimmune disorders, such as Still disease and systemic lupus erythematosus (SLE), and viral infections, indicates the possibility of hemophagocytic syndrome (Emmenegger et al., 2001), which involves the phagocytosis of red blood cells by macrophages (Knovich et al., 2009), along with a final common pathway of elevated triglycerides, ferritin, pancytopenia, and highly fatal multiple organ failure (Sekigawa et al., 2001).

Hepcidin regulates serum iron levels by activating the endocytosis and proteolysis of ferroportin, the sole mammalian iron exporter. In healthy individuals, iron status is monitored by hepatocytes, which regulate hepcidin promoter activity according to iron needs. If iron levels are low, iron is released by ferroportin, allowing hepcidin levels to remain low; if iron overload is detected, hepcidin is activated to sequester the excess iron (Ueda & Takasawa, 2018). Unregulated activity of hepcidin can therefore result in hypoferremia due to iron sequestration (Ganz & Nemeth, 2009). Interleukin-6 (IL-6), an inflammatory cytokine, stimulates hepcidin to decrease erythropoiesis due to a lack of bioavailable iron for hemoglobin (Kroot et al., 2011).

No physiologic process is present in the body to excrete excess iron, leaving individuals susceptible to developing iron overload. Iron overload may result from increased absorption, transfusion, or hereditary disease. Excess iron collects within the internal organs, specifically the liver and heart, where it causes chronic free-radical induced injury (Wang et al., 2010). Excess iron may be a symptom or complication of a hereditary disease, such as hereditary hemochromatosis (HH), an autosomal recessive disorder that causes an enhancement in the intestinal absorption of excess iron (Santos et al., 2012). Too much iron in the body can lead to a plethora of problems, including arthritis, skin pigmentation, hypogonadism, cardiomyopathy, and diabetes. The majority of individuals with HH contain mutant hemochromatosis (*HFE*) genotypes, including homozygosity for p.Cys282Tyr or p.Cys282Tyr, and compound heterozygosity for p.His63Asp; based on these results, it is suggested that genetic testing be performed for these mutations in all patients with primary iron overload and an idiopathic increase in transferrin saturation (TSAT) and/or SF values (Santos et al., 2012).

Another genetic disorder characterized by excess iron accumulation is known as neuroferritinopathy (NF). NF was first discovered in 2001 and is a movement disorder identified by excess iron in specific areas of the brain (Lehn et al., 2012). NF is the only known autosomal dominant genetic disease of neurodegeneration caused by mutations in the ferritin light polypeptide 1 (*FTL1*) gene (Keogh et al., 2013; Kumar et al., 2016). The modification causes mutant L-chain ferritins that negatively alter ferritin function and stability (Kuwata et al., 2019; McNally et al., 2019). Several conditions indicative of NF include brain iron accumulation (NBIA) disorder alongside pantothenate kinase-associated neurodegeneration (PKAN), phospholipase A_2-associated neurodegeneration, mitochondrial membrane protein-associated neurodegeneration (MPAN), and beta-propeller protein-associated neurodegeneration (BPAN) (Hayflick et al., 2018). NBIAs are typically characterized by dystonia,

Parkinsonism, spasticity, and iron accumulation within the basal ganglia. Depending on the NBIA subtype, the condition may also exhibit hyperphosphorylated tau, axonal swelling, and Lewy body formation (Arber et al., 2016). NF is typically considered as a diagnosis in patients exhibiting movement disorders, decreased SF, variable phenotypes, negative genetic testing for common movement disorders such as Huntington disease, and imaging showing potential iron deposits in the brain (Kumar et al., 2016).

Iron deficiency (ID), referring to a reduced amount of iron stores, is usually an acquired disorder that affects over one billion people worldwide (Camaschella, 2015; Miller, 2013). Inadequate iron intake is often due to poverty, malnutrition, dietary restriction, and malabsorption; additional causes include menstrual periods, gastrointestinal bleeding, and chronic blood loss (DeLoughery, 2017; Kassebaum et al., 2014; Sankaran & Weiss, 2015). SF analysis is the most efficient test for a diagnosis of ID (DeLoughery, 2017). In children, ID is most commonly caused by insufficient dietary iron intake when compared to a child's rapid growth rate, as well as gastrointestinal issues due to cow's milk (Ozdemir, 2015).

It has been reported that more than one in three pregnant women present with iron-deficiency anemia worldwide (Lewkowitz & Tuuli, 2019). Anemia in pregnant women could affect the fetus' intrauterine growth and may cause neurodevelopmental impairment (Marell et al., 2019). Maternal anemia in early pregnancy is associated with an increased risk of autism spectrum disorder, attention-deficit/hyperactivity disorder, and intellectual disability (Wiegersma et al., 2019). Efficient vitamin and mineral supplementation are vital during pregnancy for the health of both the mother and of the fetus; however, certain supplements may be more helpful than others. It has been suggested that in pregnant women, intravenous iron administration may be a more effective treatment option than oral iron administration (Lewkowitz & Tuuli, 2019).

Analytical Validity

Low SF (<30ug/L) is a sensitive and specific indicator for ID (Dignass et al., 2018). However, a normal SF level can be misleading in the context of inflammation (Peng & Uprichard, 2017). Dignass et al. (2018) published recommendations which stated that the standard ID level is <30 μg/L and that "A serum ferritin threshold of <100 μg/L or TSAT < 20% can be considered diagnostic for iron deficiency in congestive heart failure (CHF), chronic kidney disease (CKD), and inflammatory bowel disease (IBD). If serum ferritin is 100-300 μg/L, TSAT < 20% is required to confirm iron deficiency. Routine surveillance of serum ferritin and TSAT in these at-risk groups is advisable so that iron deficiency can be detected and managed (Dignass et al., 2018)."

Biomarker glycoprotein acetylation (GlycA) has been associated with chronic inflammation and utilizes nuclear magnetic resonance (NMR) to measure the serum or plasma concentration of the *N*-acetyl methyl functional groups of *N*-acetylglucosamine glycans associated with inflammation; these include transferrin, haptoglobin, α_1-acid glycoprotein, α_1-antitrypsin, and α_1-antichymotrypsin (Ritchie et al., 2015). According to Otvos et al. (2015), the simple integration of the GlycA signal to accurately quantify concentration is not possible due to signal overlap with allylic protons of unsaturated fatty acids in the plasma or serum sample; therefore, a linear least-squares deconvolution determination must be performed. In doing so, Otvos et al. (2015) have shown that GlycA has lower imprecision and variability than high-sensitivity C-reactive protein (hsCRP), cholesterol, and triglyceride testing; however, "because the GlycA signals originating from different plasma glycoproteins are not distinguishable, and the glycan

on each is heterogeneous and varies dynamically, only a rough estimate can be made of how much each contributes to measured plasma GlycA concentrations" (Otvos et al., 2015). Consequently, the GlycA test cannot be used to accurately determine concentration of individual proteins, including transferrin.

A study by Dahlfors et al. (2015) measured serum hepcidin in more than 400 patients using a competitive ELISA assay; several types of patients were included in this study including those with liver disorders and iron disorders, as well as healthy individuals. The researchers note that this ELISA assay has a good correlation with light chromatography with tandem mass spectroscopy (LC-MS/MS) (r=0.89), but it does cross-react with forms of hepcidin (hepcidin-20 and -22) that are not associated with iron disorder biomarkers (Dahlfors et al., 2015). Another study by Karlsson (2017) compared the ELISA hepcidin assay to the use of ferritin, C-reactive protein (CRP), and IL-6 to differentiate ID anemia and anemia of inflammation in elder patients. Even though the study was small (n=30), they measured a sensitivity and specificity of the hepcidin assay of 100% and 67%, respectively, as compared to the lower sensitivity but higher specificity of ferritin (91% and 83%, respectively). It was concluded that "Hepcidin shows a strong positive correlation with ferritin, and also correlates positively with C-reactive protein in this patient population (Karlsson, 2017)." Recently, Chen et al. (2019) have developed a high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) method, in accordance to CLSI-C62A guidelines, to measure serum hepcidin levels. This method has intra- and inter-day coefficients-of-variation (CVs) of <3% and <6%, respectively, with relative error rates ≤1.2% and ≤4.4% at ambient temperature and 4°C, respectively. The authors also report that the relative error rate after three cycles of freeze-thaw (-70°C) is ≤1.8% (Chen et al., 2019).

A recent study by da Silva et al. (2019) has showed that both iron deficiency anemia (IDA) and sickle cell disease (SCD) can be detected in whole human blood samples via Raman spectroscopy; this study detected both IDA and SCD, when compared to healthy subject controls, with a sensitivity of 93.8% and a specificity of 95.7%. These results were based on detailed spectra analysis methods such as partial least squares and principal component analysis (da Silva et al., 2019).

Among neonates, Gerday et al. (2020) measured urinary ferritin in neonatal intensive care unit (NICU) patients, and found that in those at risk for iron deficiency (n=49), "a corrected urine ferritin < 12 ng/mL had a sensitivity of 82% (95% CI, 67-93%) and a specificity of 100% (CI, 66-100%) for detecting iron-limited erythropoiesis, with a positive predictive value of 100% (CI, 89-100%)." Though iron deficiency can be confirmed via serum iron, transferrin, SF, among other tests, the volume of blood and costs associated with these tests necessitate a non-invasive and accurate alternative for diagnosing iron deficiency (Gerday et al., 2020).

Jones et al. (2021) investigated the effect of delayed processing on measuring 25 micronutrients and select clinical biomarkers, including iron (ferritin), in human blood samples. Blood from 16 healthy participants was collected and processed within either 2 hours or 24 hours. The concentration difference between the two process delays was compared. All analytes had a 4% or lower change in concentration between the two delays. There was no significant effect of delayed processing on ferritin. The authors concluded that "in blood collected from adult participants, delayed processing of chilled, whole blood for 24 hours did not materially affect the measured concentrations of the majority of micronutrient and selected clinical biomarkers" (Jones et al., 2021).

Bell et al. (2021) performed a meta-analysis to study genes associated with iron homeostasis. Data about blood levels of ferritin, total iron binding capacity, iron saturation, and transferrin saturation was used

from three genome-wide association studies from Iceland, the UK, and Demark. The authors identified 56 loci with variants associated with one or more of the biomarkers, 46 of which are novel variants. Specifically, "variants at *DUOX2*, *F5*, *SLC11A2* and *TMPRSS6* associate with iron deficiency anemia, while variants at *TF*, *HFE*, *TFR2* and *TMPRSS6* associate with iron overload" (Bell et al., 2021).

Clinical Validity and Utility

Dysregulated iron metabolism has been implicated in a variety of pathophysiological conditions from mild ID to anemia, iron overload, inflammation, infection, cancer, and cardiovascular and neurodegenerative diseases (Gozzelino & Arosio, 2016). Initial signs and symptoms of iron overload are insensitive and nonspecific, so laboratory studies including ferritin are clinically useful in the identification and treatment of iron overload (Fleming & Ponka, 2012; Knovich et al., 2009; Koperdanova & Cullis, 2015). According to the Hemochromatosis and Iron Overload Screening (HEIRS) study (McLaren et al., 2003), ferritin levels above 200 ng/mL (449 pmol/L) in women or 300 ng/mL (674 pmol/L) in men with no signs of inflammatory disease warrant additional testing. Therapeutic phlebotomy is indicated in patients with hemochromatosis who have high TSAT and SF levels of more than 1000 ng/mL (2247 pmol/L). Therapeutic phlebotomy is also recommended in patients who do not have anemia (Fleming & Ponka, 2012; Salgia & Brown, 2015; van Bokhoven et al., 2011). Saeed et al. (2015) used a receiver operating characteristic curve to evaluate the value of ferritin >500 ng/mL for diagnosing hemophagocytic lymphohistiocytosis (HLH) in 344 consecutive patients and found that the optimal maximum SF level for the diagnosis of HLH was 3951 ng/mL.

Abioye et al. (2019) collected data from 2,100 pregnant women in Tanzania to determine how capable hematologic biomarkers such as hemoglobin and hepcidin, were at detecting IDA in pregnant woman; hepcidin administration >1.6 µg/L was found to reduce the risk of anemia at delivery by an estimated 49%. This study suggests that both hemoglobin and hepcidin may be helpful in determining iron supplementation needs in "resource-limited countries" (Abioye et al., 2019).

A study by Ismail et al. (2019) studied the role of hepcidin in children with β-thalassemia (n = 88 total). The authors measured both serum hepcidin and SF levels as well as determined the hepcidin:ferritin ratio. As expected, serum hepcidin significantly correlated with the hepcidin:ferritin ratio, but the authors reported that there was no statistically significant difference in serum hepcidin levels between splenectomized and non-splenectomized patients. Serum hepcidin levels were more elevated in individuals with β-thalassemia, especially those with β-thalassemia major (βTM), than in control patients (21.74 ng/mL and 13.01 ng/mL, respectively). The authors conclude, "Knowing that hepcidin in serum has a dynamic and multi-factorial regulation, individual evaluation of serum hepcidin and follow up, e.g. every 6 months could be valuable, and future therapeutic hepcidin agonists could be helpful in management of iron burden in such patient (Ismail et al., 2019)."

Yuniati et al. (2019) studied the association between maternal vitamin D, ferritin, and hemoglobin levels during the first trimester of pregnancy, and how these factors affected birthweight. 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. A total of 203 Indonesian women were followed until delivery; it was determined that neither vitamin D, ferritin or hemoglobin levels significantly impacted birthweights in this study. However, the authors suggest that other unknown variables may be at play here and that nutritional supplementation during pregnancy is still important (Yuniati et al., 2019).

Kwiatek-Majkusiak et al. (2020) investigated the connection between hepcidin and chronic neuroinflammation. Serum hepcidin and IL-6 were found to be involved in the progression of Parkinson's Disease. Dysregulation in immune/inflammatory pathways, wherein levels of serum hepcidin and IL-6 would be elevated, were not only predictive of neurodegeneration, with IL-6- induced hepcidin expression in astrocytes, microglia, and epithelial cells, but also response to deep brain stimulation treatment (Kwiatek-Majkusiak et al., 2020).

Brandtner et al. (2020) found linkages between serum markers of iron metabolism and prognosis of sepsis survival. Positive correlations were found between increased serum iron and SF levels and severity of organ failure (SOFA score) and mortality. High TSAT, elevated ferritin and serum iron levels, and low transferrin concentrations were associated with decreased chances of survival as well. This indicates the utility of iron metabolism in the context of extreme systemic inflammation; from this study, it was also concluded that TSAT can be a stand-alone predictor of sepsis survival (Brandtner et al., 2020).

Nalado et al. (2020) evaluated the diagnostic validity of GDF-15 and hepcidin as biomarkers of IDA in non-dialysis CKD patients. Serum levels of GDF-15 and hepcidin were measured in 312 non-dialysis CKD patients and 184 healthy control participants in Johannesburg, South Africa. For absolute IDA diagnosis among CKD patients, GDF-15 had a predictive value of 74.02%. For functional IDA diagnosis among CKD patients, hepcidin had a predictive value of 70.1%. The authors concluded that "serum GDF-15 is a potential biomarker of absolute IDA, while hepcidin levels can predict functional IDA among CKD patients" (Nalado et al., 2020).

Phillips et al. (2021) studied how the full blood count (FBC) parameters change in older patients. FBC, mean corpuscular volume (MCV), and red cell distribution width (RDW) test results were compiled from male and female patients aged 1-100 years from the National Health Service in England. In males, the mean hemoglobin concentration increased from birth until age 20, then decreased at a steady rate from age 20 to 70, then decreased at a higher rate after age 70. In females, the mean hemoglobin concentration increased from birth until age 14, then decreased slowly from age 14 to 30, then increased again from age 30 to age 60, and then decreased after the age of 60. Overall, "hemoglobin concentrations in males and females begin to converge after age 60 and equalize by approximately 90 years." The authors concluded that FBC parameters trend throughout life, particularly "a falling hemoglobin level and rising MCV and RDW with older age" (Phillips et al., 2021).

Mei et al. (2021) performed a cross-sectional study using data from the US National Health and Nutrition Examination Survey to determine physiologically based SF concentration thresholds for iron deficiency in healthy children (12-59 months) and non-pregnant woman (15-49 years). The study analyzed the relationship between SF and hemoglobin, and the relationship between SF and soluble transferrin receptor. The study resulted in SF concentration thresholds for iron deficiency of "about 20 µg/L for children and 25 µg/L for non-pregnant women." The authors concluded that "physiologically based thresholds for iron deficiency might be more clinically and epidemiologically relevant than those based on expert opinion" (Mei et al., 2021).

Garcia-Casal et al. (2021) performed a meta-analysis studying the diagnostic accuracy of serum and plasma ferritin concentrations for detecting iron deficiency or overload in primary and secondary iron-loading syndromes. The authors used 72 studies, with a total of 6095 participants, that measured serum

or plasma ferritin concentrations. The authors compared ferritin blood tests to iron levels in the bone marrow to diagnose iron deficiency, and compared ferritin blood tests to iron levels in the liver to diagnose iron overload. The authors concluded that at a threshold of 30 µg/L, there "is low-certainty evidence that blood ferritin concentration is reasonably sensitive and a very specific test for iron deficiency." Additionally, there is "very low certainty that high concentrations of ferritin provide a sensitive test for iron overload in people where this condition is suspected." The authors note that overall confidence in the studies is low because of potential bias, indirectness, and heterogenous evidence, and that there is insufficient evidence to make conclusions about using ferritin concentrations to diagnose iron deficiency or overload in asymptomatic people (Garcia-Casal et al., 2021).

Auerbach et al. (2021) performed a study to assess the accuracy of diagnosing IDA using the complete blood cell count (CBC) and reticulocyte hemoglobin equivalent (RET-He) analysis. 556 patients referred for the diagnosis and/or treatment of anemia were studied at baseline, and 150 of the participants were later studied after intravenous iron treatment. RET-He identified iron deficiency with a 68.2% sensitivity and 69.7% specificity. RET-He predicted responsiveness to intravenous iron with 84% sensitivity and 78% specificity. The authors concluded that "CBC and RET-He can identify patients with IDA, determine need for and responsiveness to intravenous iron, and reduce time for therapeutic decisions" (Auerbach et al., 2021).

Tahara et al. (2022) examined the usage of RET-He as a marker of iron deficiency in patients with heart failure, as both anemia and iron deficiency are common among patients with heart failure. RET-He has been considered as a proxy due to the limitations of using serum ferritin and transferrin saturation for the diagnosis of iron deficiency in the clinical setting. Namely, ferritin can be overestimated in cases of chronic inflammation, such as in the case of heart failure, and thus may be inaccurately measured for the diagnosis of iron deficiency. In this prospective study, researchers enrolled 142 patients hospitalized for decompensated heart failure, with 65% of them having iron deficiency. RET-He was directly correlated with serum iron and ferritin concentrations and TSAT for iron deficiency. They found that "there was a poor relationship between quartile of RET-He and [heart failure] hospitalization or death but increases or decreases in RET-He between admission and discharge were associated with a worse outcome." This demonstrated a potential for using RET-He for predicting improvements in iron deficiency per response to IV iron and prognosis of patients with comorbid iron deficiency and heart failure.

V. Guidelines and Recommendations

Guidelines and recommendations related to the screening of anemia in certain populations are available; however, published recommendations regarding the use of ferritin as a first-line test in asymptomatic individuals have not been identified.

In regard to NF, "At present, no established guidelines or specific management recommendations for patients with NF have been identified. An individualized symptomatic approach to treatment is recommended (Kumar et al., 2016)." To date, the only NBIA guidelines published concerning diagnosis and management of the condition is pantothenate kinase-associated neurodegeneration (PKAN, formerly called Hallervorden-Spatz syndrome) (Hogarth et al., 2017).

American Gastroenterological Association (AGA)

The AGA has published its official recommendations on the gastrointestinal evaluation of iron deficiency anemia (IDA). It has stated:

1. "In patients with anemia, the AGA recommends using a cutoff of 45 ng/mL over 15 ng/mL when using ferritin to diagnose iron deficiency.
    a. In patients with inflammatory conditions or chronic kidney disease, other laboratory tests, such as C-reactive protein, transferrin saturation, or soluble transferrin saturation, may be needed in conjunction with ferritin to diagnose iron deficiency anemia (Ko et al., 2020).

American Society of Clinical Oncology (ASCO) and the American Society of Hematology (ASH)

The ASCO and ASH have published guidelines regarding the management of cancer-related anemia with erythropoiesis-stimulating agents (ESAs). It is stated that "With the exception of selected patients with MDS, ESAs should not be offered to most patients with nonchemotherapy-associated anemia. During ESA treatment, hemoglobin may be increased to the lowest concentration needed to avoid transfusions. Iron replacement may be used to improve hemoglobin response and reduce RBC transfusions for patients receiving ESA with or without ID. Baseline and periodic monitoring of iron, total iron-binding capacity, transferrin saturation, or ferritin levels is recommended" (Bohlius et al., 2019).

American Academy of Family Physicians (AAFP)

The AAPF have recommend the following with "C" evidence ratings (consensus, disease-oriented evidence, usual practice, expert opinion, or case series):

- "A low serum ferritin level is associated with a diagnosis of iron deficiency anemia,"
- "Older patients with suspected iron deficiency anemia should undergo endoscopy to evaluate for occult gastrointestinal malignancy," and
- "Low-dose formulations of iron (15 mg of elemental iron) can be effective for treatment of suspected iron deficiency anemia and have a lower risk of adverse effects than standard preparations" (Lanier et al., 2018).

Also stated is: "Patients with an elevated serum ferritin level or macrocytic anemia should be evaluated for underlying conditions, including vitamin B12 or folate deficiency, myelodysplastic syndrome, and malignancy" (Lanier et al., 2018).

The Endocrine Society

The Endocrine Society's 2018 guidelines on testosterone therapy in men with hypogonadism state, "In men deemed to have secondary hypogonadism, additional diagnostic evaluations may be needed to exclude hyperprolactinemia, head trauma, iron overload syndromes, hypothalamic or pituitary tumors, and other infiltrative or destructive hypothalamic–pituitary diseases, as well as genetic disorders associated with gonadotropin deficiency. Measuring serum prolactin and iron saturation and/or serum ferritin can help determine the presence of hyperprolactinemia and iron overload syndromes, respectively (Bhasin et al., 2018)."

The American College of Gastroenterology (ACG)

ACG practice guidelines regarding the evaluation of abnormal liver chemistries recommend that "All patients with abnormal liver chemistries in the absence of acute hepatitis should undergo testing for

hereditary hemochromatosis with an iron level, transferrin saturation, and serum ferritin [Strong recommendation, very low level of evidence] (Kwo et al., 2017).” .

World Health Organization (WHO)

The WHO guideline on the use of ferritin concentrations to assess iron status in individuals and populations, published in 2020, updated the previous serum ferritin levels recommendations. The guidelines recommend cut-off serum ferritin levels for iron deficiency in infants (0-23 months) and preschool children (24-59 months) as under 12 µg/L in apparently healthy individuals and under 30 µg/L in individuals with infections or inflammation. The guidelines recommend cut-off serum ferritin levels for iron deficiency in school age children (5-12 years), adolescents (13-19 years), adults (20-59 years), and older persons (over 60 years) as under 15 µg/L in apparently healthy individuals and under 70 µg/L in individuals with infections or inflammation. The guidelines recommend cut-off serum ferritin levels for iron deficiency in apparently healthy pregnant women in their first trimester as under 15 µg/L.

The guidelines recommend cut-off serum ferritin levels for risk of iron overload in school age children (5-12 years), adolescents (13-19 years), adults (20-59 years), and older persons (over 60 years) as over 150 µg/L in apparently healthy individuals females, over 200 µg/L in apparently healthy males, and over 500 µg/L in individuals with infections or inflammation (WHO, 2020).

International Consensus Guideline for Clinical Management of Pantothenate Kinase-Associated Neurodegeneration (PKAN)

An international group released guidelines concerning the clinical management of the NBIA condition PKAN in 2017. Although no specific recommendation is directly given regarding measurement of iron, Hogarth et al. (2017) state, “The role that iron plays in PKAN pathogenesis is still unclear because iron dyshomeostasis is a secondary phenomenon in this disorder. Nevertheless, high iron levels develop in globus pallidus and probably contribute to cell and tissue damage. The utility of iron chelators has been limited by systemic iron depletion. Newer agents more readily cross the blood-brain barrier yet have a lower affinity for iron, thereby minimizing systemic iron loss.” Concerning diagnosis of PKAN, “People suspected to have PKAN based on clinical features should undergo brain MRI using iron sensitive sequences such as SWI, GRE, T2* as a first line diagnostic investigation to identify the characteristic changes. The MRI abnormality, called the ‘eye-of-the-tiger’ sign, is observed on T2-weighted imaging and consists of hypointense signal in the globus pallidus surrounding a region of hyperintense signal (Hogarth et al., 2017).”

International Consensus Statement on the Peri-operative Management of Anemia and Iron Deficiency

An expert workshop, including a number of experienced researchers and clinicians, was conducted to develop a guidance for the diagnosis and management of anemia in surgical patients. A series of best-practice and evidence-based statements to advise on patient care with respect to anemia have been published via this workshop. It was stated that serum ferritin measurement is the most sensitive and specific test used for the identification of absolute iron deficiency (Munoz, Acheson, et al., 2017).

European Crohn's and Colitis Organisation (ECCO)

ECCO guidelines published in 2015 concerning iron deficiency and anemia in IBD with an EL-5 recommendation state, "For laboratory screening, complete blood count, serum ferritin, and C-reactive protein [CRP] should be used. For patients in remission or mild disease, measurements should be performed every 6 to 12 months. In outpatients with active disease such measurements should be performed at least every 3 months" (Dignass et al., 2015). Also mentioned in the section concerning the workup for anemia with an EL-4 recommendation is that anemia workups "should be initiated if the hemoglobin is below normal. The minimum workup includes red blood cell indices such as red cell distribution width [RDW] and mean corpuscular volume [MCV], reticulocyte count, differential blood cell count, serum ferritin, transferrin saturation [TfS], and CRP concentration. More extensive workup includes serum concentrations of vitamin B, folic acid, haptoglobin, the percentage of hypochromic red cells, reticulocyte hemoglobin, lactate dehydrogenase, soluble transferrin receptor, creatinine, and urea (Dignass et al., 2015)."

The U.S. Preventive Services Task Force (USPSTF)

The USPSTF states, "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; the current evidence is insufficient to assess the balance of benefits and harms of routine iron supplementation for pregnant women to prevent adverse maternal health and birth outcomes; the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in children ages 6 to 24 months" (USPSTF, 2015a, 2015b). All recommendations have been given a grade I.

American Society of Hematology (ASH)

In the ASH *Guidelines for Quantifying Iron Overload*, it is stated that "Despite improved availability of advanced imaging techniques, serum ferritin remains the mostly commonly used metric to monitor iron chelation therapy and remains the sole metric in many countries. Serum ferritin measurements are inexpensive and generally correlate with both total body iron stores and clinical outcomes… Given interpatient and temporal variability of serum ferritin values, serum ferritin is best checked frequently (every 3-6 weeks) so that running averages can be calculated; this corrects for many of the transient fluctuations related to inflammation and liver damage." Regarding the use of transferrin, the guidelines also state that "Iron that is bound to transferrin is not redox active, nor does it produce extrahepatic iron overload. However, once transferrin saturations exceed 85%, non-transferrin-bound iron (NTBI) species begin to circulate, creating a risk for endocrine and cardiac iron accumulation. A subset of NTBI can catalyze Fenton reactions and is known as labile plasma iron (LPI). Therefore, transferrin saturation, NTBI, and LPI are potentially attractive serum markers for iron toxicity risk. Transferrin saturation is widely available, but values cannot be interpreted if iron chelator is present in the bloodstream, so patients have to be instructed to withhold iron chelation for at least 1 day before measurement… Although some studies link elevated LPI to cardiac iron accumulation, large validation studies are lacking. Therefore, to date, these metrics remain important and interesting research tools, but are not suitable for routine monitoring" (Wood, 2014). Within the conclusion of the paper, the author notes that "Serum markers of somatic stores (ferritin and transferrin saturation) are useful surrogates for total iron stores and extrahepatic risk, respectively. However, they cannot replace LIC or cardiac T2* assessment for monitoring chelator efficacy or stratifying end organ risk" (Wood, 2014).

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI)

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (KDOQI) published guidelines in 2012. In 2013, the Kidney Disease: Improving Global Outcomes (KDIGO) group reviewed these guidelines in a separate publication. Based on the suggestions made by the KDOQI, the KDIGO "continued to recommend the use of serum ferritin concentration and transferrin saturation (TSAT) to define iron stores and iron availability. For all their imperfections, these metrics remain our best routinely available tools to assess iron status and manage iron supplementation. In the absence of superior, cost-effective, and easily applicable alternatives, this approach seems reasonable" (Kliger et al., 2013).

Further, the KDOQI stated that ferritin testing along with TSAT as part of the evaluation of iron status in individuals with chronic kidney disease who are being treated for anemia is recommended. Also, in agreement with KDIGO, the KDOQI recommend testing prior to initiation of treatment, once per month during initial treatment, and at least every 3 months after a stable hemoglobin level is reached.

Kidney Disease Improving Global Outcomes (KDIGO)

In the *2012 KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease* publication, a complete blood count, absolute reticulocyte count, serum ferritin, serum transferrin saturation (TSAT), serum vitamin $B_{12,}$ and serum folate levels are recommended as part of an initial evaluation of anemia for all CKD patients, regardless of age or stage of degree progression. Moreover, for patients undergoing ESA therapy, "including the decision to start or continue iron therapy," both TSAT and ferritin should be tested at least every 3 months; TSAT and ferritin should be tested "more frequently when initiating or increasing ESA dose, when there is blood loss, when monitoring response after a course of IV iron, and in other circumstances where iron stores may become depleted" (KDIGO, 2012).

International Society of Nephrology (ISN)

The most recent guidelines from the ISN, released in 2008, state that for CKD patients "who require iron and/or ESA therapy, measurement of serum ferritin and transferrin saturation every 1-3 months is reasonable, depending upon the clinical status of the patient, the hemoglobin response to iron supplementation, the ESA dose, and recent iron status test results; in stable patients with mild anemia (hemoglobin >110 g/l) who are not receiving iron or ESA therapy, assessment of iron status could be performed less frequently, potentially on a yearly basis" (Madore et al., 2008).

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.

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
82728	Ferritin
83540	Iron
83550	Iron binding capacity
84466	Transferrin
0024U	Glycosylated acute phase proteins (GlycA), nuclear magnetic resonance spectroscopy, quantitative Proprietary test: GlycA Lab/Manufacturer: Laboratory Corporation of America

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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DNA Ploidy Cell Cycle Analysis

Policy #: AHS – M2136	**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

S-phase fraction (SPF) is an assessment of how many cells are actively synthesizing DNA (UIHC, 2016). It is used as a measure of cell proliferation, particularly for cancer (Pinto, André, & Soares, 1999).

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. Measurement of flow cytometry-derived DNA content (DNA Index) or cell proliferative activity (S-phase fraction or % S-phase) for prognostic or therapeutic purposes in the routine clinical management of cancers **DOES NOT MEET COVERAGE CRITERIA.**

IV. Scientific Background

Cancer is the uncontrolled growth and spread of abnormal cells and is increasingly shown to be initiated, propagated, and maintained by somatic genetic events (Johnson et al., 2014). In 2020, an expected 1,806,590 Americans will be diagnosed with new cancer cases, and 606,520 Americans will die from the disease (Siegel, Miller, & Jemal, 2020).

DNA Ploidy Cell Cycle Analysis, continued

During the cell cycle, DNA synthesis is tightly regulated and only performed just as the cell is about to divide. This step of DNA replication is called the "S-phase" (Christensen, 2021). Dysfunction of DNA replication is significantly associated with cancer, and cancers frequently involve damage or removal of molecular regulators of replication (Van der Aa et al., 2013). Assessment of the fraction of cells in S-phase has been proposed as an indicator of neoplasm aggression. S-phase fraction (SPF) is thought to reflect proliferative activity of cancer and may provide prognostic or therapeutic information (Ermiah et al., 2012). Elevated proliferative activity may predict a worsened disease-free or overall survival in several cancers, such as breast, non-small cell lung, colorectal, ovarian, kidney, bladder, prostate, and endometrial cancers (Bagwell et al., 2001; Gawrychowski, Lackowska, & Gabriel, 2003; Kenney, Zieske, Rinder, & Smith, 2008; Mangili et al., 2008; Pinto et al., 2011; Ross, 1996). However, data supporting the use of SPF as a prognostic tool appears to be inconsistent at best (Locker et al., 2006). Several proprietary tests exist for the assessment of S-phase fraction. For example, NeoGenomics and GenPath both offer tests to evaluate DNA ploidy along with SPF.

Clinical Validity and Utility

Dabic et al. (2008) examined flow cytometric parameters (DNA ploidy and SPF) as predictors of survival in cervical adenocarcinoma. The authors defined proliferative activity as the sum of cells in S or G2/M phase and considered proliferative activity above 15% to be "unfavorable." The authors evaluated 51 patients from 1978 to 2004, but the *p*-value for proliferative activity was found to be 0.817, which is not statistically significant. Therefore, the authors concluded that they did not find any association of flow cytometric parameters with patient survival.

Wolfson et al. (2008) studied possible associations between measurements of DNA index (DI), S-phase fraction (SPF), and tumor heterogeneity (TH) using flow cytometry and overall survival for patients with invasive cervical carcinoma treated with definitive irradiation. The investigators examined a total of 57 patients and found 29 to have SPF under 15% and 26 above 15% (with 2 with unknown SPF). However, after a median follow-up of 3.7 years, the authors found no observable associations among DI, SPF, or TH and patient outcome. They stated that additional studies are needed to identify tumor biomarkers that could predict patients at risk for disseminated disease.

Carloni et al. (2017) evaluated the associations between SPF and peritoneal carcinomatosis from ovarian cancer. Fifty-three patients were examined, and although SPF differed among the different ploidy categories, no significant correlation was found between SPF and clinical pathological characteristics of patients. However, the authors did find that sensitivity to taxol was correlated with SPF, therefore concluding that "ploidy and SPF could facilitate the choice of therapy for patients with peritoneal carcinomatosis (Carloni et al., 2017)."

Svanvik, Stromberg, Holmberg, Marcickiewicz, and Sundfeldt (2019) examined 1113 patients diagnosed with stage I-III grade 1-3 endometrioid endometrial carcinoma in 2006-2011. They evaluated both DNA ploidy and SPF and set the SPF cutoff at 8%. The authors found that 5-year relative survival was significantly associated with SPF and DNA ploidy through a univariate statistical analysis. However, when other variables such as age, grade, and stage were added, SPF and DNA ploidy became statistically insignificant. Therefore, the authors concluded that "S-phase fraction, DNA ploidy, and p53

overexpression did not improve identification of high-risk patients by stage, grade, and age in stage I-III endometrioid endometrial carcinoma (Svanvik et al., 2019)."

Thomas et al. (2020) completed a study to analyze the prognostic implications of DNA repair, DNA ploidy and telomerase in the malignant transformation risk assessment of leukoplakia. Samples from 200 patients with oral leukoplakia, 100 patients with oral cancer and 100 healthy controls were analyzed. The DNA ploidy content was measured with high resolution flow cytometry; the authors identified that "There was significant difference in the distribution of ploidy status, telomerase activity and DNA repair capacity among control, leukoplakia and oral cancer group ($p<0.001$). When the molecular markers were compared with histological grading of leukoplakia, both DNA ploidy analysis and telomerase activity showed statistical significance ($p<0.001$) (Thomas et al., 2020)."

Taniguchi et al. investigated the correlation between flow cytometry parameters such as DNA ploidy, DNA index and S-phase fraction and clinical prognostic factors such as mitotic count and Ki-67 labelling index (LI). The cancer of interest was "gastrointestinal stromal tumours (GIST)" and eighteen specimens from laparoscopic local gastrectomy were analyzed. The authors found these flow cytometry parameters to correlate well with mitotic count ≤5 and Ki-67 LI ≤6. DNA index was found to be 83.3% accurate in predicting mitotic count ≤5 and 77.8% accurate in predicting Ki-67 LI ≤6, while S-phase fraction was found to be 94.4% accurate and 88.9% accurate, respectively. The authors concluded that "Rapid flow cytometry parameters can classify risk without the need for histological analysis." (Taniguchi et al., 2021)

V. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO) (Harris et al., 2007; Locker et al., 2006)

The ASCO's updated recommendations on the use of tumor markers in colorectal cancer state that "neither flow-cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should not be used to determine prognosis of early-stage colorectal cancer" (Locker et al., 2006). The recommendations also state that "as such, flow cytometric determination of DNA ploidy or proliferation should, at best, be considered an experimental tool" (Locker et al., 2006).

In 2007, the ASCO updated the guidelines for the use of tumor markers in breast cancer which noted that there is "insufficient evidence to support routine use in clinical practice of DNA/ploidy by flow cytometry" (Harris et al., 2007).

National Comprehensive Cancer Network (NCCN) (NCCN, 2021)

NCCN clinical practice guidelines on diagnosis and/or management of Breast Cancer (Version 2.2021), Cervical Cancer (Version 1.2021), Colon Cancer (Version 2.2021), Small Cell Lung Cancer (Version 2.2021), and Non-Small Cell Lung Cancer (Version 4.2021) do not mention cell proliferation activity (S-phase fraction or % S-phase) as a management tool (NCCN, 2021).

International Society of Gynecological Pathologists (ISGyP) Endometrial Cancer Project: Guidelines From the Special Techniques and Ancillary Studies Group (Cho et al., 2019)

These guidelines focus on biomarkers and their potential use for endometrial carcinoma.

The guideline remarks that "Other than markers which are useful in diagnosis, there are few specific studies that provide definitive evidence for the routine use of IHC [immunohistochemistry] or ploidy analysis in determining the prognosis of EC" and that "There is some literature on the association of ploidy with prognosis, with promising results, but there is a lack of definitive studies to determine its true prognostic impact".

Overall, the guideline states that "Clearly, large prospective, well defined, uniform studies are needed to determine the possible role of IHC for specific biomarkers and ploidy analysis in the clinical setting." (Cho et al., 2019)

VI. State and Federal Regulations (as applicable)

Numerous FDA-approved tests exist for the assessment of SPF. 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
88182	Flow cytometry, cell cycle or DNA analysis

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

Epithelial Cell Cytology in Breast Cancer Risk Assessment

Policy #: AHS – G2059	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 1/8/24 (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

Nipple aspiration and/or ductal lavage are non-invasive techniques to obtain epithelial cells for cytological examination to aid in the evaluation of nipple discharge for breast cancer risk (Golshan, 2022). Fine needle aspiration (FNA) is another approach that can be used in the initial diagnosis of a suspicious breast mass, although core biopsy is superior in sensitivity, specificity, and correct histological grading (Moy et al., 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.

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. Cytologic analysis of epithelial cells to assess breast cancer risk and manage patients at high risk of breast cancer **DOES NOT MEET COVERAGE CRITIERIA**.

III. Scientific Background

Breast cancer is the most frequently diagnosed cancer and ia leading cause of cancer death in the United States. Nipple discharge is a common breast complaint. Most nipple discharge is of benign origin; however, it is necessary to differentiate patients with benign nipple discharge from those who have an underlying pathology. In approximately 5-15 percent of pathologic nipple discharge cases, cancer is identified (Golshan, 2020).

Breast cancer originates in breast epithelium and is associated with progressive molecular and morphologic changes. Individuals with atypical breast ductal epithelial cells have an increased relative risk of breast cancer. Cytological evaluation of epithelial cells in nipple discharge has been used as a diagnostic aid. Due to the scant cellularity of specimens obtained by expression or aspiration of nipple discharge, ductal lavage was developed to enhance the ease and efficiency of collecting breast epithelial cells for cytologic analysis. The analysis of breast-specific liquid biopsies, such as nipple aspirate fluid, has potential to be used as a biomarker profiling technique for monitoring breast health (Shaheed et al., 2018). Researchers report that the measurement of nipple aspirate fluid, including miRNA, pathological nipple discharge, and breast ductal fluids, may help to improve early detection and management of breast cancer (Moelans et al., 2019).

Fine needle aspiration (FNA) is a biopsy option for a suspicious palpable breast mass. FNA is a rapid diagnosis technique, but it is not as accurate as core needle biopsy. FNA cannot differentiate in situ and invasive cancer and has higher rates of negative results and insufficient samples than core needle biopsy. The success of FNA results also varies with the operator and cytopathologist (Joe & Esserman, 2023).

Analytic Validity

In a retrospective study of 618 patients with nipple discharge over a 14-year period, the sensitivity and specificity of cytology were 17 and 66 percent, respectively; the authors concluded that “nipple discharge cytology has little complementary diagnostic value” (Kooistra et al., 2009).

Clinical Utility and Validity

Hornberger et al. (2015) performed a meta-analysis on the use of nipple aspirate fluid (NAF) in identifying breast cancer based on proliferative epithelial disease (PED). The authors reviewed 16 articles, 20808 unique aspirations, and 17378 subjects. Among cancer-free patients, 51.5% aspirations contained fluid, of which 27.7% showed a PED on cytology. Of the two prospective studies of 7850 women, patients with abnormal cytology showed a 2.1-fold higher risk of developing breast cancer compared to those without fluid (Hornberger et al., 2015).

Chatterton et al. (2016) measured sex steroid levels in nipple aspirate fluid; hormones were measured in samples from 160 breast cancer cases and 157 controls. Results showed a significantly higher concentration of dehydroepiandrosterone (DHEA) in the nipple aspirate fluid of patients with breast cancer compared to controls; further, DHEA levels were highly correlated with estradiol levels, indicating “a potentially important role of this steroid in breast cancer risk” (Chatterton et al., 2016).

Kamalı and Kamalı (2022) studied the usefulness of testing methods in surgical decision making. The study included 141 patients with pathological nipple discharge who were planning to undergo surgery. The diagnostic efficiency of ductal lavage cytology was compared to that of ultrasonography, mammography,

magnetic resonance imaging, and ductography. The sensitivity of ductal lavage cytology was 70.5% and the specificity was 94.1%. The authors conclude that "negative cytology does not exclude the possibility of malignancy, and positive results do not help in the differential diagnosis" (Kamalı & Kamalı, 2022).

IV. Guidelines and Recommendations

American Society of Breast Surgeons (ASBS)

The Official Statement by the American Society of Breast Surgeons (ASBS, 2019) regarding Screening Mammography does not mention ductal lavage at all in their statement.

In 2016, the ASBS published a consensus guideline on the concordance assessment of image-guided breast biopsies and the management of borderline or high-risk lesions. These guideline state that "The decision to excise a papillary lesion without atypia needs to be individualized based on risk, including such criteria as size; symptomatology, including palpability and presence of nipple discharge; and breast cancer risk factors" (ASBS, 2016). This is the only mention of nipple discharge in the document.

National Comprehensive Cancer Network (NCCN)

National Comprehensive Cancer Network Clinical Practice Guidelines in breast cancer screening and diagnosis (NCCN, 2023) state that "thermography and ductal lavage are not recommended by the NCCN Panel for breast cancer screening or diagnosis." The NCCN also notes that "the FDA has issued a safety alert stating that ductal lavage should not be a replacement for mammograms" (NCCN, 2023).

Food and Drug Administration (FDA)

In 2017 the FDA issued a safety warning (FDA, 2017) stating that "…the FDA is unaware of any valid scientific data to show that a nipple aspirate test, when used on its own, is an effective screening tool for any medical condition, including the detection of breast cancer or other breast disease."

American College of Radiology (ACR)

The 2022 ACR appropriateness criteria for the evaluation of nipple discharge do not mention cytology. The ACR states that "image-guided FNA and core biopsy are not required for the evaluation of physiologic nipple discharge" but "image-guided FNA and core biopsy are not required for the evaluation of physiologic nipple discharge". The ACR also notes "although some institutions demonstrate good results using FNA, larger series have shown that core biopsy is superior to FNA in terms of sensitivity, specificity, and correct histologic grading of a lesion" (Sanford et al., 2022).

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
88108	Cytopathology, concentration technique, smears and interpretation (e.g., Saccomanno technique)
88112	Cytopathology, selective cellular enhancement technique with interpretation (e.g., liquid based slide preparation method), except cervical or vaginal
88172	Cytopathology, evaluation of fine needle aspirate; immediate cytohistologic study to determine adequacy for diagnosis, first evaluation episode, each site
88173	Cytopathology, evaluation of fine needle aspirate; interpretation and report
88177	Cytopathology, evaluation of fine needle aspirate; immediate cytohistologic study to determine adequacy for diagnosis, each separate additional evaluation episode, same site (List separately in addition to code for primary 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.

VII. Evidence-based Scientific References

ASBS. (2016). *Consensus Guideline on Concordance Assessment of Image-Guided Breast Biopsies and Management of Borderline or High-Risk Lesions*. https://www.breastsurgeons.org/docs/statements/Consensus-Guideline-on-Concordance-Assessment-of-Image-Guided-Breast-Biopsies.pdf

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VIII. Revision History

Revision Date	Summary of Changes
1/8/24	Coverage criteria #1 updated to reflect that all cytological analysis for breast cancer diagnosis do not meet coverage criteria, as biopsy should be used to diagnose. Now reads: "Cytologic analysis of epithelial cells to assess breast cancer risk and manage patients at high risk of breast cancer **DOES NOT MEET COVERAGE CRITERIA**."

Epithelial Cell Cytology in Breast Cancer Risk Assessment

Policy #: AHS – G2059	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 1/8/24 (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

Nipple aspiration and/or ductal lavage are non-invasive techniques to obtain epithelial cells for cytological examination to aid in the evaluation of nipple discharge for breast cancer risk (Golshan, 2022). Fine needle aspiration (FNA) is another approach that can be used in the initial diagnosis of a suspicious breast mass, although core biopsy is superior in sensitivity, specificity, and correct histological grading (Moy et al., 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.

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. Cytologic analysis of epithelial cells to assess breast cancer risk and manage patients at high risk of breast cancer **DOES NOT MEET COVERAGE CRITIERIA**.

Evaluation of Dry Eyes

Policy #: AHS – G2138	**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

Dry eye disease (dysfunctional tear syndrome, DED) is defined by the Dry Eye Workshop II as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles" (Craig, Nichols, et al., 2017). 5-15% of the United States population suffers from dry eye disease, leaving a substantial burden on functional vision, general health status, and workplace productivity (Dana, Meunier, Markowitz, Joseph, & Siffel, 2020).

II. Related Policies

Policy Number	Policy Title
AHS-M2083	Genetic Testing for Ophthalmologic 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. Testing of tear osmolarity in patients suspected of having dry eye **MEETS COVERAGE CRITERIA** to aid in determining the severity of dry eye disease as well as monitor effectiveness of therapy.
2. Testing for MMP-9 protein in human tears **DOES NOT MEET COVERAGE CRITERIA** to aid in the diagnosis of patients suspected of having dry eye disease based on comprehensive eye examination.

3. Testing for lactoferrin and/or IgE to aid in the diagnosis of patients suspected of having dry eye disease **DOES NOT MEET COVERAGE CRITERIA**.
4. All other testing used in the diagnosis of patients suspected of having dry eye disease **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Tears are necessary for maintaining the health of the inner and outer surfaces of the eyelid and for providing clear vision. The tear film of the eye consists of aqueous, mucous, and lipid components. A healthy tear film is necessary for protecting and moisturizing the cornea, as well as for providing a refracting surface for light entering the eye (Willcox et al., 2017). Dysfunction of any component of the tear film can lead to dry eye disease (dysfunctional tear syndrome, DED). Dry eye is a common and often chronic problem, particularly in older adults as age affects the entire lacrimal functional unit (Ezuddin, Alawa, & Galor, 2015). The exact prevalence of dry eye is unknown due to difficulty in defining the disease and the lack of a single diagnostic test to confirm its presence, but the 2013 National Health and Wellness Survey estimated the rate of dry eye in the United States to be 6.8%, or about 16.4 million people; prevalence tended to increase with age, with the 18-34 age group only comprising 2.7% of the total and the 75+ age group comprising 18.6% (Farrand, Fridman, Stillman, & Schaumberg, 2017; Shtein, 2020). Risk factors for dry eye include increasing age, systemic comorbidities such as diabetes and autoimmune disease, and therapeutic treatments for anxiety, depression, and sleep disorders (Periman, 2020).

Further, the 2017 Tear Film & Ocular Surface (TFOS) Society International Dry Eye Workshop (DEW) II reported that “the core mechanism of dry eye disease is tear hyperosmolarity, which is the hallmark of the disease” (Craig, Nichols, et al., 2017).

Dry eye is classified into two general groups: decreased tear production and increased evaporative loss. Decreased tear production may lead to hyperosmolarity of the tear film and inflamed ocular surface cells. An age-related ductal obstruction is the most common cause of decreased tear production. Increased evaporative loss is typically caused by problems in the Meibomian gland when the glands that produce the lipid portion of the tear film fail. This lipid portion normally allows the tear film to spread evenly, minimizing evaporation. In both groups, tear film hyperosmolarity and subsequent ocular surface inflammation lead to the variety of symptoms and signs associated with dry eye (Shtein, 2020).

Most patients will present with symptoms of chronic eye irritation, such as red eyes, light sensitivity, blurred vision, or unusual sensations (gritty, burning, foreign, etc.). However, significant variability in the patient-reported symptoms and signs, as well as a lack of correlation between these symptoms and signs, make it difficult to diagnose dry eye, and no single definitive test to diagnose dry eye exists. Dry eye is typically diagnosed with a combination of patient symptoms and physical findings, such as reduced blink rate or eyelid malposition (Shtein, 2020). Incomplete blinking may also be considered for mild-to-moderate dry eye assessment (Jie, Sella, Feng, Gomez, & Afshari, 2019). Further, visual acuity was found to be particularly poor in those with vision-related symptoms due to dry eyes (Szczotka-Flynn et al., 2019).

Evaluation of Dry Eyes, continued

The primary way to treat dry eye is artificial tears, although corticosteroids, topical cyclosporine A, or anti-inflammatories such as Lifitegrast ophthalmic solution 5% may be used to supplement treatment. Avoiding environmental factors, such as heavy smoke or dry heating air, is also recommended (Messmer, 2015). It was recently reported by Holland, Darvish, Nichols, Jones, and Karpecki (2019), who reviewed two decades worth of data on the safety and efficacy of controlled topical ophthalmic drug administration for DED treatment, that poor standardization of endpoints across studies causes challenges in the improvement of this field. However, recent advances in drug delivery and a greater understanding of DED will assist in the improvement of ophthalmic drugs.

Accurate diagnosis of dry eye disease requires a variety of tests including patient-reported symptom questionnaires, tear film break-up time (TFBUT), Schirmer test, ocular surface staining, and meibomian gland functionality. However, many of these tests lack consistency and reliability in diagnosis. New tools have been developed which allow for the quantification of tear film characteristics including measurement of tear osmolarity and measurement of inflammatory mediators such as matrix metallopeptidase enzymes, and biomarkers such as lactoferrin (Shtein, 2020).

Tear Osmolarity

Osmolarity is a measurement of the concentration of dissolved solutes in a solution. Hyperosmolarity of the tear film is a recognized and validated marker of dry eye. The following tear osmolarity thresholds have been suggested for establishing the severity of dry eyes: 270-308 mOsm/L for normal eyes, 308-316 mOsm/L for mild dry eye, and >316 mOsm/L for moderate to severe dry eye (Milner et al., 2017). Tomlinson, Khanal, Ramaesh, Diaper, and McFadyen (2006) suggested a cut-off of 316 mOsm/L, but the sensitivity was found to be 0.59 when applied to the independent sample described in the study. Furthermore, decreasing the cut-off to increase the sensitivity decreased the specificity and overall accuracy significantly. Overall, the overlap between normal and dry eyes contributes heavily to the difficulty in establishing a cut-off (Tomlinson et al., 2006). Some studies suggest that osmolarity shows the strongest correlation with severity of dry eye based on the metrics used, but at the same time lack correlation to other objective signs of dry eye. In general, tear osmolarity results vary between clinical signs and symptoms, which can make them difficult to interpret (Akpek et al., 2018).

The test “TearLab” is based on assessment of the osmolarity of tears. TearLab collects a 50 µL tear sample, analyzes its electrical impedance, and provides an assessment of the osmolarity of the sample and thereby the tear (Willcox et al., 2017). Baenninger et al. (2018) completed an extensive systematic review investigating 1362 healthy eyes of participants from 33 different studies; this review found a weighted mean osmolarity of 298 mOsm/L via the TearLab test. Final comments from the researchers highlighted the great variability of osmolarity measurements that were found with the TearLab system, suggesting caution when interpreting TearLab osmolarity results (Baenninger et al., 2018).

Brissette, Drinkwater, Bohm, and Starr (2019) measured the utility of the TearLab test in 100 patients with DED-like symptoms who had normal tear osmolarity results. This study aimed to use the test to identify diagnoses other than DED. All patients included in the study had a normal tear osmolarity test (<308 mOsm/L in each eye, and an inter-eye difference <8 mOsm/L). The researchers report that “A possible alternate diagnosis was established in 89% of patients with normal tear osmolarity testing. The

most frequent diagnoses included anterior blepharitis (26%) and allergic conjunctivitis (21%)" (Brissette et al., 2019). This highlights the utility of the TearLab test to differentiate between DED and other eye disorders with overlapping symptoms.

In a retrospective study by Tashbayev et al., 757 patients diagnosed with symptomatic dry eye disease (DED) were recruited to investigate the clinical utility of tear osmolarity measurement. The TearLab osmometer was used to measure osmolarity in both eyes and the results were compared to Ocular Surface Disease Index (OSDI), tear film break-up time (TFBUT), ocular surface staining (OSS), Schrimer test, and meibomian gland functionality tests. According to their data, TearLab results were not significantly different between the healthy controls and the DED patients. Many studies confirm that tear osmolarity greater than 308 MOsm/mL indicates a loss of homeostasis in the tear, therefore, is used as a cut-off value. Many of the healthy controls had tear osmolarity levels above the recommended cut-off value of 308 mOsm/L, and a substantial proportion of the diagnosed DED patients had tear osmolarity levels below the cut-off value. In the DED patient group, osmolarity levels in the right and left eye were 275–398 mOsm/L and 272–346 mOsm/L, respectively. In the control group, osmolarity levels in the right and left eyes were 281–369 mOsm/L and 275–398 mOsm/L, respectively. Therefore, the authors suggest that "tear osmolarity measured with TearLab osmometer cannot be used as a key indicator of DED (Tashbayev et al., 2020)."

As shown in the above studies, there have been issues in the past regarding the use of tear osmolarity as a diagnostic tool. First, no criteria for the measurement of osmolarity have been established. Studies reviewing osmolarity as a diagnostic tool do not use uniform numbers in their calculations (e.g. no uniform cut-off values, no standardized severity measures, etc.). To compound this issue, high variance in osmolarity due to outside factors, such as sleep deprivation, altitude, or even whether the right or left eye was used to produce the tears, can occur. This difficulty in establishing osmolarity ranges has caused an overlap between the ranges of healthy and dry eye osmolarity. Although measuring fluctuations between osmolarity readings has been suggested as a diagnostic (caused by increased instability), the line between healthy eyes and dry eyes is blurred (Willcox et al., 2017). However, a recent report by the TFOS DEWS II states that tear osmolarity "is a global, early stage marker of the disease and has been shown to be able to effectively track therapeutic response and inform the clinician as to whether there has been a loss of tear film homeostasis" (Craig, Nichols, et al., 2017).

Matrix Metallopeptidase (MMP) Enzymes

Inflammation is a common factor across the subtypes of DED. Levels of inflammatory mediators, such as cytokines, may be assessed in the tear film. For example, the matrix metallopeptidase (MMP) enzymes play an important role in wound healing and inflammation by degrading collagen. Elevated levels of MMP-9, a member of the MMP family produced by corneal epithelial cells (Chotikavanich et al., 2009; Honda et al., 2010), have been observed in the tears of patients with dry eye (Sambursky et al., 2013). A study with 101 patients with DED and controls (54 controls, 47 with DED) was performed to assess correlation of the protein MMP-9 with dry eye. All 101 underwent MMP-9 testing of the tear film and were evaluated for symptoms and signs of DED. The tear film was then analyzed for MMP-9 by *InflammaDry*, which detects MMP-9 levels of more than 40 ng/mL. The MMP-9 results were positive in

19 of the 47 dry eye patients (40.4%) and 3 of the 54 controls (5.6%). The authors concluded that "MMP-9 correlated well with other dry eye tests and identified the presence of ocular surface inflammation in 40% of confirmed dry eye patients," and suggested it may be helpful to identify patients with autoimmune disease and ocular surface inflammation (Messmer, von Lindenfels, Garbe, & Kampik, 2016). The American Academy of Ophthalmology (AAO) has noted MMP-9 does not differentiate dry eye from any other inflammatory ocular surface disease and does not include this test in its appendix on diagnostic tests (Akpek et al., 2018).

Chan, Ye, Chan, Chu, and Jhanji (2016) aimed to assess the utility of MMP-9 measurement in patients with post-laser-assisted in situ keratomileusis (LASIK) dry eyes compared to aged-matched controls. The *InflammaDry* was used to measure MMP-9 levels in tear film. Results showed that "The tear film MMP-9 levels were 52.7±32.5 ng/mL in dry eyes and 4.1±2.1 ng/mL in normal eyes ($p<0.001$). MMP-9 levels were >40 ng/mL in 7/14 (50.0%) post-LASIK dry eyes. The InflammaDry was positive in 8/14 (57.1%) post-LASIK eyes. All positive cases had tear film MMP-9 levels ≥38.03 ng/mL. Agreement between InflammaDry and MMP-9 was excellent with Cohen κ value of 0.857 in post-LASIK dry eyes" (Chan et al., 2016). However, only half of the post-LASIK patients with dry eyes exhibited significant inflammation with heightened levels of MMP-9 (Chan et al., 2016).

A cross-sectional study by Jun JH (2020) investigated if the tear volume in dry eye disease (DED) patients affects the results of the MMP-9 immunoassay (*Inflammadry*). 188 DED patients were enrolled in the study. Positive MMP-9 tests were confirmed in 120 patients, and negative results were noted in 68 patients. However, the authors observed that with a small sample volume, the reliability of the test result was impaired. The manufacturer also pointed out that less than 6 µl of sample volume could produce false-negative results. In this study, patients with higher tear volumes showed higher band densities, but subjects with lower tear volumes showed lower band densities on the immunoassay. In conditions such as Sjögren syndrome that present with markedly decreased tear secretion, *Inflammadry* could display negative results despite the elevated tear MMP-9 concentration. In addition, "among the participants of the present study, a strong positive band was identified even in patients with mild or nearly no fluorescein staining of the cornea and conjunctiva, who are expected to have very mild inflammatory eye surface inflammation (Jun JH, 2020)." In conclusion, this study determined the volume dependency of the MMP-9 immunoassay, which could induce false-negative results clinically (Jun JH, 2020).

Lactoferrin

Another biomarker associated with inflammation is lactoferrin. Lactoferrin is thought to promote the healing process resulting from inflamed dry eyes and is used to assess the lacrimal glands (Willcox et al., 2017). The test "TearScan" from Advanced Tear Diagnostics (ATD) uses this biomarker to assess dry eye, listing a sensitivity of 83%, a specificity of 98%, and a coefficient of variation of <9% (ATD, 2016b). However, lactoferrin's sensitivity for dry eye discrimination was assessed to be 44.2% by a third party review (Versura, Bavelloni, Grillini, Fresina, & Campos, 2013). TearScan uses a quantitative immunoassay to assess lactoferrin and requires a 0.5 µL tear sample. TearScan also offers a similar test assessing the amount of immunoglobulin E (IgE) in tears, purporting that the test can identify any "allergic component

of dry eye etiologies"; the sample report lists a sensitivity of 93%, a specificity of 96%, and a coefficient of variation of <9%, but no other studies corroborated these numbers (ATD, 2016a).

A meta-analysis was performed to highlight the potential role of tear lactoferrin as a diagnostic biomarker for dry eye disease (DED). All original studies reporting an estimate of the average lactoferrin concentration in healthy subjects and those affected by DED were searched. A pooled mean difference of 0.62 (95% CI, 0.35–0.89) in lactoferrin concentration was observed in DED patients, showing a significant decrease in lactoferrin concentrations in the tears of subjects affected by DED. A study reported that administration of lactoferrin protein in mice led to a decrease in oxidative damage and an enhancement of tear function (Kawashima et al., 2012). Lastly, the author notes that "to compare data across studies and to validate lactoferrin as a diagnostic biomarker, there is still a need for further development of standardized protocols of tear collection, processing and storage (Ponzini, Scotti, Grandori, Tavazzi, & Zambon, 2020)."

Additional Tests

Other tests noted by the American Academy of Optometry (AAO) are the tear break-up time test, the ocular surface dry staining test, the Schirmer test, and the fluorescein dye disappearance test. The tear break-up time test evaluates the precorneal tear film's stability with a fluorescein dye, which is inserted in the lower eyelid. If the tear film layer develops a dark discontinuity (usually blue) in under 10 seconds, the result is considered abnormal. The ocular surface dry staining test stains areas of discontinuity of the corneal epithelial surface, which may contribute to dryness. A fluorescein dye is typically used, although a rose bengal dye or a lissamine green dye may be used as well. The Schirmer test quantifies the amount of tears produced by each eye. This is done by placing small strips filter paper in the lower eyelid and checking the length (in mm) of wet strips in a certain amount of time. This test is noted as an extremely variable test, so it should not be used as the only diagnostic test. Finally, the fluorescein dye disappearance test places a certain amount of fluorescein dye on the ocular surface, and then evaluates how much of that dye was cleared from the surface (Akpek et al., 2018; Shtein, 2020).

Evaluation of dry eyes is difficult for numerous reasons. Currently, no "gold standard" or globally accepted guideline for diagnosis of dry eye exists, and no threshold between healthy and affected eyes has been established. Many other features of testing (repeatability, high variability, including highly variable sensitivity and specificity of tests and dependence on clinical conditions) and the disease itself—its multifactorial status, examiner subjectivity, reliance on patient-based questionnaires, for example—make diagnosis of dry eye especially challenging (Kanellopoulos & Asimellis, 2016). Despite promising sensitivities, specificities, or other strong statistical findings, these numbers should still be considered in the context of clinical findings (Akpek et al., 2018).

V. Guidelines and Recommendations

Dysfunctional Tear Syndrome (DTS) Panel (Milner et al., 2017)

A study assessed the new diagnostic techniques and treatment options for DED and associated tear film disorders. Experts from the Cornea, External Disease, and Refractive Society (DTS Panel) convened by the study found examining tear osmolarity useful in diagnosis "in combination with other clinical

assessments and procedures." The same panel also stated that the use of MMP-9 may only be valid for more severe cases of dry eye since the diagnostic test is only positive past 40 ng/mL. The panel recommended that osmolarity be evaluated before any ocular surface assessment, then an evaluation of ocular inflammation can be done, and finally a Schirmer strip test should be done (Milner et al., 2017).

American Academy of Ophthalmology (AAO) (Akpek et al., 2018)

The AAO states "no single test is adequate for establishing the diagnosis of dry eye" and recommends that the combination of findings from diagnostic tests can be useful to understanding a patient's condition. In particular, the AAO states, "tests results should be considered within the context of symptoms and other clinical findings. Rather than relying solely on a single measure of tear osmolarity, correlation with clinical findings or differences in osmolarity over time or under different conditions is more informative for confirming the diagnosis of dry eye. Indeed, most recent studies confirm that normal subjects have exceptionally stable tear film osmolarity, whereas tear osmolarity values in dry eye subjects become unstable quickly and lose homeostasis with environmental changes. These data reinforce the long-held belief that tear film instability due to increased evaporation of tears resulting in hyperosmolarity (i.e., evaporative dry eye) is a core mechanism of the disease" (Akpek et al., 2018). The guideline covers the currently used diagnostic tests, which are as follows: assessment of tear osmolarity, MMP-9, tear production, fluorescein dye or tear function index, tear break up time, ocular surface dye staining, and lacrimal gland function (Akpek et al., 2018).

The following table is provided by Akpek et al. (2018):

Table 2 Characteristic Findings for Dry Eye Disease Diagnostic Tests

Test	Characteristic Findings
Tear osmolarity	Elevated; test-to-test variability; inter-eye differences considered abnormal
Matrix metalloproteinase-9	Indicates presence of inflammation which dictates treatment
Aqueous tear production (Schirmer test)	10 mm or less considered abnormal
Fluorescein dye disappearance test/tear function test	Test result is compared with a standard color scale
Tear break-up time	Less than 10 seconds considered abnormal
Ocular surface dye staining	Staining of inferior cornea and bulbar conjunctiva typical
Lacrimal gland function	Decreased tear lactoferrin concentrations

Tear Film & Ocular Surface (TFOS) Society (Craig, Nelson, et al., 2017; Craig, Nichols, et al., 2017)

The TFOS society held the International Dry Eye Workshop II in 2017. From this workshop, the society published recommendations on the management and treatment of DED. The authors state that when diagnosing DED, it is important to distinguish between the type (aqueous deficient dry eye or

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evaporative dry eye) and to determine the underlying etiology as this is crucial for proper management (Craig, Nelson, et al., 2017). These guidelines also stated that "neurotrophic keratopathy accompanied by neuropathic pain and symptoms should definitely be considered in differential diagnosis of patients with intense symptoms despite mild signs (Craig, Nelson, et al., 2017)."

Regarding diagnostic testing, the TFOS states that any patient who obtains a positive score on the Dry Eye Questionnaire-5 or Ocular Surface Disease Index should be subject to a clinical examination. "The presence of any one of three specified signs; reduced non-invasive break-up time; elevated or a large interocular disparity in osmolarity; or ocular surface staining (of the cornea, conjunctiva or lid margin) in either eye, is considered representative of disrupted homeostasis, confirming the diagnosis of DED. If a patient has DED symptoms and their practitioner does not have access to all these tests, a diagnosis is still possible, based on a positive result for any one of the markers, but may require referral for confirmation if the available homeostasis markers are negative (Craig, Nelson, et al., 2017)." After confirmation with any of the aforementioned tests (i.e. reduced non-invasive break-up time <10 seconds, an elevated or large interocular disparity in osmolarity ≥308 m0sm/L in either eye or an interocular difference > 8 m0sm/L, or ocular surface staining including > 5 corneal spots, > 9 conjunctival sports, or a lid margin ≥ 2mm in length and ≥ 25% in width), further evaluation should be conducted including meibography, lipid interferometry, and tear volume measurement to assess severity and help determine the best treatment plan (Craig, Nelson, et al., 2017).

Further, the consensus recommendation from the society on tear osmolarity testing states, "The low variation of normal subjects contributes to the high specificity of the marker and makes it a good candidate for parallelization and therapeutic monitoring. Accordingly, normal subjects don't display elevated osmolarity, so a value over 308 mOsm/L in either eye or a difference between eyes >8 mOsm/L are good indicators of a departure from tear film homeostasis and represent a diseased ocular surface" (Craig, Nichols, et al., 2017).

Regarding MMP-9 testing, the guidelines state that "With the availability of newer immunosuppressive medications and trials concerning these drugs it is logical that inflammation should be assessed. The exact modality used may need to be varied depending on the pathway or target cell upon which the immunosuppressive drug acts, and such diagnostic tools should be used for refining patient selection as well as monitoring after commencement of treatment. Costs of these diagnostic tests should be considered, but these should be calculated from a holistic standpoint. For example, if the tests can assist the channeling of patients to appropriate healthcare services there may be cost savings for reduced referrals" (Craig, Nichols, et al., 2017).

American Optometric Association (AOA, 2010)

The AOA published consensus-based clinical practice guidelines for care of a patient with ocular surface disorders. These guidelines note that there is a "lack of a defined diagnostic test or protocol and a lack of congruity between patient symptoms and clinical tests." The AOA also notes that the condition itself is ill defined and that dry eye is often a symptom of another condition such as blepharitis or another glandular dysfunction (AOA, 2010). There have not been any updates on this topic from the AOA since this 2010 statement.

Consensus Guidelines for Management of Dry Eye Associated with Sjögren Disease (Foulks et al., 2015)

In 2015, clinical guidelines for management of dry eye associated with Sjögren disease were published by a consensus panel which evaluated reported treatments for DED. The recommendations state, "Evaluation should include symptoms of both discomfort and visual disturbance as well as determination of the relative contribution of aqueous production deficiency and evaporative loss of tear volume. Objective parameters of tear film stability, tear osmolarity, degree of lid margin disease, and ocular surface damage should be used to stage severity of dry eye disease to assist in selecting appropriate treatment options. Patient education with regard to the nature of the problem, aggravating factors, and goals of treatment is critical to successful management. Tear supplementation and stabilization, control of inflammation of the lacrimal glands and ocular surface, and possible stimulation of tear production are treatment options that are used according to the character and severity of dry eye disease" (Foulks et al., 2015). Further, tear osmolarity was identified as the testing method with the highest level of evidence for all DED related tests.

American Society of Cataract and Refractive Surgery (ASCRS) Cornea Clinical Committee (Starr et al., 2019)

American Society of Cataract and Refractive Surgery (ASCRS) released guidelines to aid surgeons in diagnosing visually significant ocular surface disorders (OSD) before refractive surgery. The ASCRS Cornea Clinical Committee recommends initial screening procedures including ASCRS Standard Patient Evaluation of Eye Dryness (SPEED) II questionnaire, tear osmolarity, and matrix metalloproteinase (MMP-9) testing. If any of the three initial screening tests are abnormal, the patient is at risk for ocular surface disease, and additional diagnostic tests can be performed to determine dry eye sub-type. Non-invasive tests are recommended to minimize disruption to the ocular surface, cornea, and tear film. These tests include tear lipid layer thickness, noninvasive tear breakup time (NIKBUT), tear meniscus height, meibography, topography, tear lactoferrin levels, and measures of optical scatter. However, these tests are not essential to the fundamental algorithm.

The ASCRS also notes a point of care test that assesses lactoferrin levels (TearScan). The guideline notes its three proprietary biomarkers which are as follows: "salivary protein-1 (SP-1, immunoglobulin A [IgA], immunoglobulin G [IgG], immunoglobulin M [IgM]); (2) carbonic anhydrase-6 (CA-6, IgA, IgG, IgM); and (3) parotid secretory protein (PSP, IgA, IgG, IgM)". The authors comment that this test can be used to detect Sjögren syndrome early. However, the authors also note that "no member of the ASCRS Cornea Clinical Committee has used it [TearScan] in clinical practice" (Starr et al., 2019).

VI. State and Federal Regulations (as applicable)

On December 3, 1993, the FDA approved the lactoferrin microassay system by Touch Scientific, Inc (FDA, 1993). Lactoferrin diagnostic kits are commercially available options for tear film biomarkers (Willcox et al., 2017).

On May 14, 2009, the FDA approved *TearLab* created by Ocusense Inc. From the FDA site: this device is used "to measure the osmolality of human tears to aid in the diagnosis of patients with signs or symptoms of DED, in conjunction with other methods of clinical evaluation" (TearLab, 2009).

On November 20, 2013, the FDA approved *InflammaDry* created by Rapid Pathogen Screening Inc. From the FDA site: "*InflammaDry* is a rapid, immunoassay test for the visual, qualitative in vitro detection of elevated levels of the MMP-9 protein in human tears from patients suspected of having dry eye to aid in the diagnosis of dry eye in conjunction with other methods of clinical evaluation. This test is intended for prescription use at point-of-care sites" (FDA, 2013).

A search of "dry eye" on the FDA devices webpage on 01/14/2021 yielded 2 results relevant to DED diagnostic testing. 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
82785	Gammaglobulin (immunoglobulin); IgE
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
83861	Microfluidic analysis utilizing an integrated collection and analysis device, tear osmolarity

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

G2138 Evaluation of Dry Eyes

Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota Transplant Testing

Policy #: AHS – G2060	**Prior Policy Name & Number (as applicable):** AHS – G2060 – Fecal Analysis in the Diagnosis of Intestinal Dysbiosis
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

Intestinal dysbiosis is defined as a disruption or imbalance of the intestinal microbial ecology (Guinane & Cotter, 2013). Dysbiosis is associated with many diseases, including irritable bowel syndrome (IBS), inflammatory bowel diseases (IBD), obesity, allergy, and diabetes (Carding et al., 2015; Marietta et al., 2020).

II. Related Policies

Policy Number	Policy Title
AHS-G2056	Diagnosis of Idiopathic Environmental Intolerance
AHS-G2061	Fecal Calprotectin Testing
AHS-G2121	Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease

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.

Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota Transplant Testing, continued

1. Prior to fecal microbiota transplant (FMT), fecal analysis by culture for the following microorganisms **MEETS COVERAGE CRITERIA:**
    a. Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*
    b. Vancomycin-resistant *Enterococci* (VRE)
    c. Carbapenem-resistant *Enterobacteriaceae* (CRE)
    d. Methicillin-resistant *Staphylococcus aureus* (MRSA)
    e. *Campylobacter*
    f. *Shigella*
    g. *Salmonella*
2. Prior to fecal microbiota transplant (FMT), fecal analysis for the following microorganisms by nucleic acid amplification testing (NAAT) **MEETS COVERAGE CRITERIA.**
    a. *Clostridium Difficile*
    b. Campylobacter
    c. *Salmonella*
    d. *Shigella*
    e. Shiga toxin-producing *Escherichia coli*
    f. Norovirus
    g. Rotavirus
    h. COVID-19 (SARS-CoV-2)
3. Prior to fecal microbiota transplant (FMT), fecal analysis for the following microorganisms by nucleic acid amplification testing (NAAT) **DOES NOT MEET COVERAGE CRITERIA**:
    a. Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*
    b. Vancomycin-resistant *Enterococci* (VRE)
    c. Carbapenem-resistant *Enterobacteriaceae* (CRE)
    d. Methicillin-resistant *Staphylococcus aureus* (MRSA)
    e. Any other microorganisms not listed above

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. As a diagnostic test for the evaluation of intestinal dysbiosis, irritable bowel syndrome, malabsorption, or small intestinal overgrowth of bacteria, fecal analysis of the following components **DOES NOT MEET COVERAGE CRITERIA:**
    a. Triglycerides

b. Chymotrypsin
c. Iso-butyrate, iso-valerate, and n-valerate
d. Meat and vegetable fibers
e. Long chain fatty acids
f. Cholesterol
g. Total short chain fatty acids
h. The levels of *Lactobacilli*, bifidobacteria, and *E. coli* and other "potential pathogens," including *Aeromona*, *Bacillus cereus*, *Campylobacter*, *Citrobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *S. aureus*, *Vibrio*
i. For the identification and quantitation of fecal yeast (including *C. albicans*, *C. tropicalis*, *Rhodoptorul* and *Geotrichum*)
j. N-butyrate
k. Beta-glucoronidase
l. pH
m. Short chain fatty acid distribution (adequate amount and proportions of the different short chain fatty acids reflect the basic status of intestinal metabolism)
n. Fecal secretory IgA

IV. Scientific Background

The human intestinal tract has a diverse and complex microbial community necessary for health and nutrition. The gut microbiome is estimated to consist of upwards of 1000 bacterial species (Guinane & Cotter, 2013; Ley et al., 2010). The microbiota functions with the immune system to protect against pathogens. It also performs essential metabolic functions, extracting certain forms of energy and nutrients from food and providing a source of other essential nutrients and vitamins (Carding et al., 2015).

The gut is colonized at birth, but the intestinal microbiome changes rapidly during the first year of life. In adults, each individual's unique population of gut microbiota is fairly stable over time; however, alterations in the microbiota can result from exposure to various environmental factors, including diet, toxins, drugs, and pathogens (Carding et al., 2015; Lozupone et al., 2012; Snapper & Abraham, 2022). This change in an individual's normal microbiota is called "dysbiosis" (Johnston Jr., 2021). Dysbiosis has been associated with obesity (Ley et al., 2009) malnutrition (Kau et al., 2011), systematic diseases such as diabetes (Qin et al., 2012) and chronic inflammatory diseases such as inflammatory bowel disease (IBD) (Frank et al., 2007; Guinane & Cotter, 2013). Both direct assessment of the gut microbiota (examination of bacteria levels) and indirect assessment (measurement of non-living markers such as pH or beta-glucoronidase) have been proposed for investigation of intestinal dysbiosis.

Microbial or microbial-derived components have also been cited as potential representations of dysbiosis. For example, short-chain fatty acids have been identified as a mechanism to regulate intestinal processes and, as such, may represent dysbiosis (Johnston Jr., 2021). These fatty acids are the products of bacterial fermentation of fiber, and the concentrations of these fatty acids have been noted to decrease in IBD cases. Some fatty acids, especially butyrate, have been demonstrated to factor in signaling cascades that control immune function, which indicates a role in controlling intestinal inflammation (Parada Venegas et al., 2019). Ongoing research has uncovered many other potential links between intestinal metabolism and gut microbiota so many markers have been suggested as potential indicators of dysbiosis.

Many tests exist for the assessment of the gut microbiome. Due to the amount of conditions associated (or proposed to be associated) with gut microbiome balance, there are many corresponding tests, including screening measures intended for completely healthy individuals. These tests primarily revolve around nucleic acid amplification; microbial DNA or RNA is obtained from the sample, unique sequences are identified, and the nucleic acid is quantified (Raby, 2020). For instance, Viome offers a comprehensive screening panel that measures “all microorganisms” in the gut (including viruses, archaea, yeast, fungi, parasites, and bacteriophages). Those measurements are combined into a score for various issues, such as inflammatory activity, digestive efficiency, methane gas production, overall gas production, and more (Viome, 2019b). Viome also provides a list of nutritional recommendations, broken down into individual foods. Viome performs RNA sequencing with Illumina NextSeq and uses bioinformatics algorithms to classify taxonomic data (Viome, 2019a).

Some companies may offer companion products with their gut microbiome tests. BioHM provides a similar assessment of bacterial and fungal species in an individual’s gastrointestinal tract, but the company also offers a series of probiotics. These probiotics are intended for various purposes, such as colon cleansing or immunity (BioHM, 2018). Other companies offering a gut microbiome test include Thryve, GenCove, DayTwo, American Gut, and Genova (DNATestingChoice, 2019; Genova, 2019).

The potential clinical impact of imbalance in the intestinal microbiota suggests a need for standardized diagnostic methods to facilitate microbiome profiling. Documenting dysbiosis has traditionally relied on classical microbiological techniques and the ability to culture pure isolates for identification and classification; however, the ability to classify bacteria and archaea according to individual 16S rRNA sequences can now possibly provide a rapid and detailed means of profiling complex communities of microorganisms (Casen et al., 2015; Zoetendal et al., 1998). Laboratory analysis of various fecal biomarkers have also been proposed as a method of identifying individuals with intestinal dysbiosis and may be useful in providing insight into the role of intestinal health and disease, and the development of non-gastrointestinal conditions associated with intestinal dysbiosis. However, there is a current lack of literature on the normal ranges of these biomarkers, which limit the applicability of these analyses in a general clinical setting (Bäckhed et al., 2012; Berry & Reinisch, 2013; Pang et al., 2014).

A technique revolving around restoring balance in a patient’s microbiome is fecal microbiota transplantation (FMT). FMT is the infusion of stool from a healthy donor to a patient with presumed gut dysbiosis. The concept behind this technique is that the healthy donor’s stool can facilitate a restoration of the ill patient’s gut microbiome. This technique has seen some significant success in the treatment of *C. difficile* infections and may have potential applications in some other gastrointestinal or metabolic conditions such as IBD or IBS. As with any transplant procedure, there are several screening procedures

that must be undertaken to minimize risk of infection or other disease transmission. These screening procedures include evaluation of donor history, serum testing, and stool testing. The pathogens screened for in the donor's stool sample may vary between institutions, although some pathogens are universally screened for (such as enteric pathogens). (Kim & Gluck, 2019)

Clinical Utility and Validity

Falony et al. (2016) analyzed "two independent, extensively phenotyped cohorts: the Belgian Flemish Gut Flora Project (FGFP; discovery cohort; N = 1106) and the Dutch LifeLines-DEEP study (LLDeep; replication; N = 1135)." These two sets were integrated with global data sets, combining to yield 3948 items. A "core" set of 14 genera was identified. 69 clinical and questionnaire-based covariates were found to be associated with microbiota compositional variation with a 92% replication rate. The authors noted that "stool consistency showed the largest effect size, whereas medication explained largest total variance and interacted with other covariate-microbiota associations, but early-life events such as birth mode were not reflected in adult microbiota composition" (Falony et al., 2016)

Zhernakova et al. (2016) sequenced the gut microbiomes of 1,135 participants from a Dutch population-based cohort. The authors identified relations between the microbiome and "126 exogenous and intrinsic host factors, including 31 intrinsic factors, 12 diseases, 19 drug groups, 4 smoking categories, and 60 dietary factors." "Significant" associations were found between the gut microbiome and various intrinsic, environmental, dietary, medication parameters, and disease phenotypes. The authors calculated that 18.7% of variation in microbial composition could be explained by these factors, and they observed that fecal chromogranin A was exclusively associated with 61 microbial species, totaling 53% of the microbial composition. A more diverse microbiome was associated with low CgA concentrations. The authors concluded that "these results are an important step toward a better understanding of environment-diet-microbe-host interactions" (Zhernakova et al., 2016).

Lo Presti et al. (2019) profiled the fecal and mucosal microbiota of IBD and IBS patients. 38 IBD patients, 44 IBS patients, and 47 healthy controls were included, and overall, 107 fecal samples were provided. The authors found that "*Anaerostipes* and *Ruminococcaceae* were identified as the most differentially abundant bacterial taxa in controls, *Erysipelotrichi* was identified as [a] potential biomarker for IBS, while *Gammaproteobacteria*, *Enterococcus*, and *Enterococcaceae* [were identified] for IBD" (Lo Presti et al., 2019).

Malham et al. (2019) investigated the microbiotic profile of pediatric IBD. 143 IBD patients and 34 healthy controls were included. A reduced "richness" in microbiotic profile was observed in IBD patients compared to healthy controls. In ulcerative colitis (UC), that reduced richness was associated with high intestinal inflammation and extensive disease. Nine species were "significantly" associated with a healthy microbiome, and three species were associated with IBD. The authors remarked that the microbiome composition could differentiate between Crohn's Disease, UC, and healthy controls (Malham et al., 2019).

Danilova et al. (2019) compared the gut microbiome composition of IBD patients to healthy controls. 95 IBD patients and 96 healthy controls were included. The authors noted an increase of Proteobacteria and Bacteroidetes bacteria and decrease of Firmicutes bacteria and Euryarchaeota archaea in IBD

patients. Butyrate-producing and hydrogen-utilizing bacteria were observed to have lower representation in IBD patients. Short-chain fatty acids (SCFA) were also found to have a lower absolute content in IBD patients. The authors suggested that this finding may "indicate inhibition of functional activity and number of anaerobic microflora and/or an [*sic*] change in SCFA utilization by colonocytes" (Danilova et al., 2019).

Vaughn et al. (2018) in reviewing the current status of intestinal dysbiosis and fecal transplantation found that "it is hypothesized that intestinal dysbiosis may contribute to the pathogenesis of many diseases, especially those involving the gastrointestinal tract. Therefore, fecal microbiota transplantation (FMT) is increasingly being explored as a potential treatment that aims to optimize microbiota composition and functionality (Vaughn et al., 2018)." Holleran et al. also found that fecal transplant is not recommended for use outside of *Clostridium difficile* infection (CDI) due to concerns regarding outcome and safety; however, several case series and randomized controlled trials have described its use in a research environment for a few gastrointestinal conditions related to intestinal dysbiosis, including ulcerative colitis (UC), Crohn's disease (CD) and irritable bowel syndrome (IBS). The most successful reports of the clinical efficacy of FMT in gastrointestinal conditions outside of CDI have been in treating UC (Holleran et al., 2018).

Costello et al. (2019) evaluated fecal microbiota transplantation (FMT)'s efficacy on inducing remission in ulcerative colitis (UC). The authors compared anaerobically prepared donor FMT (n = 38) to autologous FMT (stool provided by patient themselves, n = 35). The primary outcome was defined as "steroid-free remission of UC… a total Mayo score of ≤2 with an endoscopic Mayo score of 1 or less at week 8." A total of 69 patients completed the trial, with the primary outcome being achieved in 12 of 38 donor FMT patients, compared to 3 of 35 receiving autologous FMT. Five of the 12 patients achieving the primary outcome in the "donor cohort" maintained remission at 12 months. The authors concluded that "in this preliminary study of adults with mild to moderate UC, 1-week treatment with anaerobically prepared donor FMT compared with autologous FMT resulted in a higher likelihood of remission at 8 weeks. Further research is needed to assess longer-term maintenance of remission and safety" (Costello et al., 2019).

Myneedu et al. (2019) performed a meta-analysis to evaluate whether fecal microbiota transplantation (FMT) was successful in treating IBS. A total of 8 single-arm trials (SATs, 90 patients total) and 5 randomized controlled trials (RCTs, 151 patients, 105 controls) were included. In the SAT cohort, the authors identified 59.5% of IBS patients demonstrating a significant improvement. In the RCT cohort, there were no significant differences between treatment and control cohorts, either by the IBS Severity Scoring System or the IBS Quality of Life (IBS-QOL). The authors concluded that "FMT was not effective in IBS. Variations in FMT methods and patient factors may contribute to the heterogeneous results of the trials" (Myneedu et al., 2019).

In a prospective survey-based study, Saha et al. (2021) studied the long-term safety profile of fecal microbiota transplantation (FMT) for recurrent C. *difficile* infection (CDI). 609 patients who underwent FMT were contacted at 1 week, 1 month, 6 months, 1 year and greater than 2 years after transplantation. Symptoms and new medical diagnosis were recorded at each time point. Less than 1 year after FMT, greater than 60% of patients had diarrhea and 19-33% had constipation. At 1 year, 9.5% of patients reported additional CDI episodes. Additionally, patients with IBD, dialysis dependent kidney disease, and multiple FMTs had a higher risk of diarrhea. When patients were followed up after 2 years post-FMT, 73 new diagnoses were reported including gastrointestinal disorders (13%), weight gain (10%), and new

infections unrelated to FMT (11.8%). The median time for new infections post-FMT was 29 months. The authors conclude that FMT "appears safe with low risk of transmission of infections. Several new diagnoses were reported, which should be explored in future studies" (Saha et al., 2021).

In a 12-week double-blind placebo-controlled pilot trial, (Yu et al., 2020) studied the use of FMT to improve metabolic outcomes in obese patients. From a total of 24 patients, 12 adults with obesity and mild to moderate insulin resistance were given weekly oral FMT capsules from healthy lean donors and 12 adults were given. At 0, 6, and 12 weeks, various metabolic parameters were measured including HbA1c, body weight, body composition, and resting energy expenditure. According to the results, there were no significant differences between the two groups in glycemic outcomes, weight, or body composition over the 12-week period. There was a minor improvement in HbA1c after FMT as compared to placebo. These results suggest "that intestinal microbial manipulation by FMT capsules does not meaningfully alter human metabolism and weight in adults with obesity" (Yu et al., 2020).

Macareño-Castro et al. (2022) conducted a systematic review on the use of FMT on Carbapenem-resistant Enterobacteriaceae. In using 10 studies with a combination of both retrospective and prospective cohorts, they found that among 112 FMT recipients with confirmed CRE, 78.7% of patients experienced CRE decolonization at the end of study follow-up (6-12 months). The predominant strains reported were *Klebsiella pneumoniae* and *Escherichia coli*. The researchers also reported that there were no “severe complications even in immunosuppressed patients and in those with multiple underlying conditions.” This overall supports the clinical utility of FMT for CRE, but requires more studies, such as randomized trials, to validate the safety and reliable use for complete bacterial eradication.

V. Guidelines and Recommendations

World Gastroenterology Organization Global Guidelines

The WGO published guidelines on functional gastrointestinal (GI) symptoms. In it, they identify diagnostic tests for these symptoms. The basic diagnostic tests are as follows:

- Complete blood cell count (CBC)
- Erythrocyte sedimentation rate (ESR) / C-reactive protein (CRP)
- Biochemistry panel
- Fecal occult blood (patient aged > 50 y)
- Pregnancy test
- Liver function tests
- Calprotectin or other fecal test to detect inflammatory bowel disease in patients thought to have IBS, but in whom inflammatory bowel disease (IBD) is a possibility; now routine in many primary care settings (in the United Kingdom)
- Celiac serology; considered routine in areas with a high prevalence of celiac disease
- Stool testing for ova and parasites (Hunt et al., 2014)

The WGO also released their global guidelines for Inflammatory Bowel Disease in 2015 (published in 2016). Their recommendations concerning stool examination and testing are as follows:

- "Routine fecal examinations and cultures should be carried out to eliminate bacterial, viral, or parasitic causes of diarrhea."
- "Testing for *Clostridium difficile* (should be considered even in the absence of antecedent antibiotics) — should be carried out within 2 hours of passage of stools."
- "A check for occult blood or fecal leukocytes should be carried out if a patient presents without a history of blood in the stool, as this can strengthen the indication for lower endoscopy. Where lower endoscopy is readily available, these tests are rarely indicated."
- "Lactoferrin, α1-antitrypsin. The main reason for listing this test is to rule out intestinal inflammation, rather than using it as a positive diagnostic test. It may not be available in developing countries, but it can be undertaken relatively inexpensively and easily with rapid-turnaround slide-based enzyme-linked immunoassay (ELISA) tests."
- "Calprotectin — a simple, reliable, and readily available test for measuring IBD activity — may be better for UC than CD; the rapid fecal calprotectin tests could be very helpful in developing countries. If available, a home test may be useful as a routine for follow-up (Bernstein et al., 2016)."

American Gastroenterological Association (AGA)

The AGA published a review to "describe key principles in the diagnosis and management of functional gastrointestinal (GI) symptoms in patients with inflammatory bowel disease". In it, they include the following relevant items:

- "Alternative pathophysiologic mechanisms should be considered and evaluated (small intestinal bacterial overgrowth, bile acid diarrhea, carbohydrate intolerance, chronic pancreatitis) based on predominant symptom patterns."
- "Until further evidence is available, fecal microbiota transplant should not be offered for treatment of functional GI symptoms in IBD."
- "In a recent cross-sectional analysis, no association was observed between IBS symptoms and microbiome alterations among patients with IBD although effects of confounding could not be excluded." (Colombel et al., 2019)The AGA published guidelines on FMT, including information on donor pathogen screening. *C. difficile* toxin B and culture for enteric pathogens were "suggested" to be screened for, *Giardia*, *Cryptosporidium*, *Isospora* and *Cyclospora*, *Listeria*, *E. coli* O157, *Vibrio*, and *Norovirus* should be "considered", and Cytomegalovirus, Human T-cell lymphoma virus, Epstein–Barr virus, *Dientamoeba fragilis*, *Blastocystis hominis*, *Strongyloides stercoralis*, *Entamoeba histolytica*, *H. pylori*, *Schistosoma*, JC virus, Vancomycin-resistant *enterococci*, and Methicillin-resistant *Staphylococcus aureus* should "maybe" [term used by authors] be screened (Kelly et al., 2015).

American College of Gastroenterology (ACG)

The ACG published a guideline regarding the management of Crohn's Disease. In it, they recommend that "In patients who have symptoms of active Crohn's disease, stool testing should be performed to

include fecal pathogens, *Clostridium difficile* testing, and may include studies that identify gut inflammation such as a fecal calprotectin" (Lichtenstein et al., 2018).

The ACG published a guideline regarding management of ulcerative colitis. In it, the ACG writes that "FMT requires more study and clarification of treatment before use as a therapy for UC [ulcerative colitis]." The ACG comments that the variability across all steps of the procedure (donor screening, delivery, treatment duration, et al.) makes interpretation of the current results "difficult". Finally, the ACG notes that some institutions have been using "comprehensive intestinal pathogen testing through PCR-based assays that include many bacterial and viral pathogens," but that the "prevalence and impact of non–C. diff intestinal pathogens detected through such assays remain to be robustly established" (Rubin et al., 2019).

ACG published a guideline regarding management of irritable bowel syndrome. ACG does not recommend the use of fecal transplant for the treatment of global IBS symptoms. "Evidence to support FMT for the treatment of IBS is limited and of very low quality and thus cannot be recommended at present" (Lacy et al., 2021).

ACG published a guideline regarding use of FMT in recurrent and severe C. *difficile* infection. ACG suggests considering FMT for "patients with severe and fulminant CDI refractory to antibiotic therapy, in particular, when patients are deemed poor surgical candidates. For patients experiencing their second or further recurrence of CDI, FMT can be delivered to prevent further recurrences through capsule or colonoscopy. Enema may be used if other methods are unavailable." ACG suggests "repeat FMT for patients experiencing a recurrence of CDI within 8 weeks of an initial FMT. FMT should be considered for recurrent CDI in patients with IBD" (Kelly et al., 2021).

European Crohn's and Colitis Organization (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:

- "At diagnosis, every patient should have a biochemical assessment with full blood count, inflammatory markers (C-reactive protein [CRP])… and a stool sample for microbiological analysis, including *C. difficile*."
- "Stool specimens should be obtained to exclude common pathogens and specifically assayed for C difficile toxin." (Maaser et al., 2018)

2012 Rome Foundation Report

An international Working Group convened in 2012 "to provide clinical guidance on modulation of gut microbiota in IBS" and released their findings on intestinal microbiota in functional bowel disorders: a Rome foundation report in 2013. They state the following "Diagnostic and therapeutic general recommendations":

- "There is currently no clinically useful way of identifying whether the microbiota are disturbed in particular patients with irritable bowel syndrome (IBS).

- Dietary evaluation and exclusion of possible sources of unabsorbable carbohydrates including fermentable oligo-, di- and mono-saccharides and polyols and excessive fibre could be beneficial in select patients.
- Probiotics have a reasonable evidence base and should be tried, for a period of at least 1 month, at adequate doses before a judgement is made about the response to treatment.
- The utility of testing for small intestinal bacterial overgrowth (SIBO) in the setting of IBS remains an area of uncertainty.
- If SIBO is strongly suspected based on clinical presentation and testing is being considered, using stringent criteria for the glucose breath test or jejunal aspirate appear to be the best tests.
- Consideration should be given to discontinuing proton pump inhibitors in those with SIBO.
- There is emerging evidence that non-absorbable antibiotics may have the potential to reduce symptoms in some patients with IBS (Simren et al., 2013)."

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases (ESPGHAN/ESPID)

These joint guidelines reviewed management of acute gastroenteritis (AGE) in children. In it, they note that AGE does not require a specific diagnostic workup and that "microbiological investigation is not helpful in most cases." Fecal markers are also not recommended for differentiating viral and bacterial AGE. However, the guidelines observe that "microbiological investigations may be considered in children with underlying chronic conditions (e.g., oncologic diseases, IBDs, etc), in those in extremely severe conditions, or in those with prolonged symptoms in whom specific treatment is considered" (Guarino et al., 2014).

National Institute for Health and Care Excellence (NICE, 2017)

NICE updated their IBS guidelines in 2017. In it, they list the following items about diagnostic tests:

"In people who meet the IBS diagnostic criteria, the following tests should be undertaken to exclude other diagnoses:

- full blood count (FBC)
- erythrocyte sedimentation rate (ESR) or plasma viscosity
- c-reactive protein (CRP)
- antibody testing for coeliac disease (endomysial antibodies [EMA] or tissue transglutaminase [TTG]).

The following tests are not necessary to confirm diagnosis in people who meet the IBS diagnostic criteria:

- ultrasound
- rigid/flexible sigmoidoscopy
- colonoscopy; barium enema
- thyroid function test
- faecal ova and parasite test
- faecal occult blood

- hydrogen breath test (for lactose intolerance and bacterial overgrowth)" (NICE, 2017).

British Society of Gastroenterology (BSG)

The BSG published a guideline on the investigation of chronic diarrhoea in adults. Relevant items include:

- For malabsorption, fecal tests have not received "significant support" in publications and have not "established themselves in clinical practice outside specialist centres".
- "We suggest culture of small bowel aspirates as it is the most sensitive test for small bowel bacterial overgrowth (SBBO), but methods are poorly standardized and positive results may not reflect clinically significant SBBO… in the absence of an optimal test to confirm the presence of bacterial overgrowth and in those with a high test probability of SBBO, we recommend an empirical trial of antibiotics; the value of this approach has not been subject to definitive study."
- "We recommend faecal elastase testing as the preferred non-invasive test for pancreatic function" (Arasaradnam et al., 2018).

The BSG also published an extensive guideline on the management of Inflammatory Bowel Disease (including both ulcerative colitis (UC) and Crohn's disease) in adults. Their relevant comments and recommendations include:

- "In patients presenting with suspected UC, stool cultures and *Clostridium difficile* toxin assay should always be performed to rule out infective causes."
- "Ileocolonoscopy with biopsy is established as the first-line investigation for suspected Crohn's disease."
- "We recommend that all patients presenting with acute flares of colitis should have stool cultures for enteroinvasive bacterial infections and stool *Clostridium difficile* assay."
- "In spite of these encouraging data, FMT [Faecal microbial transplantation] remains an investigational treatment for use only in clinical trials in IBD."
- "There is currently no place for FMT in the management of IBD unless complicated by *C. difficile* infection outside of the clinical trial setting" (Lamb et al., 2019)

British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS)

This joint guideline was published to provide guidance on "the use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications." These guidelines include a list of items that should be screened for potential stool donors, which are as follows:

- "*Clostridium difficile* PCR"
- *"Campylobacter*, *Salmonella*, and *Shigella* by standard stool culture and/ or PCR"
- "Shiga toxin-producing *Escherichia coli* by PCR"
- "Multi-drug resistant bacteria, at least CPE [*carbapenemase-producing Enterobacteriaceae*] and ESBL [extended spectrum beta-lactamase]"
- "Stool ova, cysts and parasite analysis, including for Microsporidia"

- "Faecal antigen for *Cryptosporidium* and *Giardia"*
- "Acid fast stain for *Cyclospora* and *Isospora"*
- *"Helicobacter pylori* faecal antigen"
- "Norovirus, rotavirus PCR."

The above list is for stool screening. A separate list is provided for serum screening. The guideline also recommends that "donors should have successfully completed a donor health questionnaire and laboratory screening assays both before and after the period of stool donation" (Mullish et al., 2018).

Infectious Diseases Society of America/American College of Gastroenterology/American Society for Gastrointestinal Endoscopy/American Gastroenterological Association/North American Society for Pediatric Gastroenterology, Hepatology and Nutrition

These joint guidelines were sent to the FDA regarding recurrent Clostridium difficile infection (CDI). In it, the guidelines recommend screening donors for fecal microbiota transplantation (FMT) for C. difficile toxin B and performing a culture for enteric pathogens (IDSA/ACG/ASGE/AGA/NASPGHAN, 2013).

NASPGHAN published an FMT guideline for children in 2019, and the same analytes for screening (C difficile toxin B, culture for enteric pathogens) were recommended (Davidovics et al., 2019).

An addendum was published to the 2019 guidelines due to the 2019 FDA Safety Warning regarding FMT. In it, the following recommendation was made: "FMT donor stool screening should include (but not be limited to) MDRO testing for spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococci* (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), and methicillin-resistant *Staphylococcus aureus* (MRSA). Donors and/or stools positive for MDROs should not be used for FMT" (Michail et al., 2020).

Food and Drug Administration (FDA)

The FDA has issued a guidance statement for fecal microbiota transplant (FMT) stating that it will exercise enforcement discretion regarding the investigational new drug (IND) requirements for the use of fecal microbiota for transplantation. In 2019, the FDA updated their guidance on FMT, stating that "FMT donor stool testing must include MDRO testing to exclude use of stool that tests positive for MDRO. The MDRO tests should at minimum include extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), and methicillin-resistant Staphylococcus aureus (MRSA). Culture of nasal or peri-rectal swabs is an acceptable alternative to stool testing for MRSA only. Bookend testing (no more than 60 days apart) before and after multiple stool donations is acceptable if stool samples are quarantined until the post-donation MDRO tests are confirmed negative (FDA, 2019)."

In an April 2020 update, the FDA addressed the topic of fecal microbiota transplantation within the context of the 2020 COVID-19 outbreak. The FDA included additional protections regarding stool donation and donor screening, which are as follows:

- "Stool donor screening, including an assessment of whether, since December 1, 2019, the donor was diagnosed with laboratory-confirmed SARS-CoV-2 infection, experienced symptoms of COVID-19 (e.g., fever, cough, shortness of breath) not explained by another diagnosis, or was exposed to a suspected or confirmed case of COVID-19 or SARS-CoV-2 infection."
- "Testing of the stool donation or stool donor for SARS-CoV-2 virus or RNA. Testing approaches might include testing upper respiratory specimens (e.g., nasal swabs) or other specimens (e.g., rectal swabs or stool donations)." (FDA, 2020a)

Fecal Microbiota Transplantation Workgroup (2011)

This Working Group published guidelines on FMT. Fecal donor screening recommendations were included. The following analytes were recommended to be screened:

- "*C difficile* toxin B by PCR; if unavailable, then evaluation for toxins A and B by enzyme immunoassay (EIA)
- Routine bacterial culture for enteric pathogens
- Fecal Giardia antigen
- Fecal Cryptosporidium antigen
- Acid-fast stain for Cyclospora, Isospora, and, if antigen testing unavailable, Cryptosporidium
- Ova and parasites
- *Helicobacter pylori* fecal antigen (for upper gastrointestinal [GI] routes of FMT administration)" (Bakken et al., 2011).

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
82239	Bile acids; total
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
82705	Fat or lipids, feces; qualitative
82710	Fat or lipids, feces; quantitative
82715	Fat differential, feces, quantitative
82725	Fatty acids, nonesterified
82784	Gammaglobulin (immunoglobulin); IgA, IgD, IgG, IgM, each

Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota Transplant Testing, continued

83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83630	Lactoferrin, fecal; qualitative
83986	pH; body fluid, not otherwise specified
84311	Spectrophotometry, analyte not elsewhere specified
87045	Culture, bacterial; stool, aerobic, with isolation and preliminary examination (e.g., KIA, LIA), salmonella and shigella species
87046	Culture, bacterial; stool, aerobic, additional pathogens, isolation and presumptive identification of isolates, each plate
87075	Culture, bacterial; any source, except blood, anaerobic with isolation and presumptive identification of isolates
87076	Culture, bacterial; anaerobic isolate, additional methods required for definitive identification, each isolate
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87081	Culture, presumptive, pathogenic organisms, screening only
87102	Culture, fungi (mold or yeast) isolation, with presumptive identification of isolates; other source (except blood)
87106	Culture, fungi, definitive identification, each organism; yeast
87177	Ova and parasites, direct smears, concentration and identification
87209	Smear, primary source with interpretation; complex special stain (e.g., trichrome, iron hemotoxylin) for ova and parasites
87328	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; cryptosporidium
87329	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; giardia
87336	Infectious agent antigen detection by immunoassay technique, (e.g., enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; entamoeba histolytica dispar group
87493	Infectious agent detection by nucleic acid (DNA or RNA); Clostridium difficile, toxin gene(s), amplified probe technique
87500	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin resistance (e.g., enterococcus species van A, van B), amplified probe technique
87641	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, methicillin resistant, amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
89160	Meat fibers, feces
S3708	Gastrointestinal fat absorption study

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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Fecal Calprotectin Testing in Adults

Policy #: AHS – G2061	**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

Calprotectin is a small calcium-binding protein found in high concentration in the cytosol of neutrophils (Fagerhol, Dale, & Andersson, 1980) and to a lesser extent monocytes and macrophages (Hsu et al., 2009). Active intestinal inflammation and disturbance of the mucosa results in entrance of neutrophils (containing calprotectin) into the lumen and subsequent excretion in feces. Detection of fecal calprotectin is used to distinguish inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS) and other causes of abdominal discomfort, bloating, and diarrhea (Walsham & Sherwood, 2016).

II. Related Policies

Policy Number	Policy Title
AHS-G2060	Fecal Analysis In The Diagnosis Of Intestinal Dysbiosis
AHS-G2121	Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease
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) For the differential diagnosis between non-inflammatory gastrointestinal disease (e.g., IBS) and inflammatory gastrointestinal disease (e.g., IBD), fecal calprotectin testing **MEETS COVERAGE CRITERIA**.

2) For the monitoring of gastrointestinal conditions such as inflammatory bowel disease (IBD) **or** to assess for response to therapy or for relapse, fecal calprotectin testing **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 all other conditions not mentioned above, fecal calprotectin testing **DOES NOT MEET COVERAGE CRITERIA**.

IV. Scientific Background

Inflammatory bowel disease (IBD) includes several chronic, immune-mediated inflammatory gastrointestinal disorders; the most common of these disorders are Crohn's disease and ulcerative colitis (Boirivant & Cossu, 2012). On the other hand, irritable bowel syndrome (IBS), another gastrointestinal disorder, is a non-inflammatory condition. These disorders often share similar symptoms including abdominal discomfort, pain, bloating, and diarrhea (Burri & Beglinger, 2014). An estimated two thirds of Americans have experienced these IBS and/or IBD symptoms (Almario et al., 2018). Differentiating gastrointestinal tract symptoms due to IBS from those due to residual inflammation from IBD is challenging (Gibson, 2019; Halpin & Ford, 2012). However, the detection of fecal calprotectin can be used to effectively distinguish between these conditions (Walsham & Sherwood, 2016).

Calprotectin is a small calcium- and zinc-binding protein. This protein is primarily detected in monocytes and macrophages. During active intestinal inflammation, neutrophils migrate to the mucosa, damaging the mucosal structure. This causes leakage of these neutrophils and therefore calprotectin into the lumen and eventually the feces. Calprotectin is homogenously distributed in feces, is stable up to 7 days at room temperature, and correlates well with the "gold standard" of the indium-labeled leukocyte test (Walsham & Sherwood, 2016).

Fecal calprotectin is now accepted as one of the most useful tools to assist with the clinical management of IBD, although the optimal cut-off laboratory value for both differentiating IBD from IBS and managing IBD may vary depending on clinical settings (Maaser et al., 2019; Mumolo et al., 2018). A value of 50 µg/g is quoted by the majority of manufacturers of calprotectin kits (Tibble, Sigthorsson, Foster, Forgacs, & Bjarnason, 2002). In a young patient, a cutoff of 150 µg/g is recommended. As fecal calprotectin is increased in gastroenteritis associated with viral or bacterial infection, a value between 50 µg/g and 150 µg/g should always be repeated 2-3 weeks later (Walsham & Sherwood, 2016).

Fecal calprotectin is typically measured with polyclonal or monoclonal antibodies that detect various features on the protein structure; these tests may be quantitative or qualitive. Manufacturers of this type of test include Calpro and Bühlmann (Walsham & Sherwood, 2016).

Clinical Utility and Validity

Fecal calprotectin is increasing in utilization for the evaluation of IBD (Higuchi & Bousvaros, 2020). Meta-analyses of fecal calprotectin by both von Roon et al. (2007) and van Rheenen et al. (2010) found an overall sensitivity and specificity for IBD of >90%. Waugh et al. (2013) also completed a meta-analysis as part of the national Health Technology Assessment program which found a pooled sensitivity of 93% and specificity of 94% when distinguishing between IBS and IBD in adults with a fecal calprotectin cut-off of 50 µg/g.

Molander et al. (2012) evaluated fecal calprotectin levels after induction therapy with TNFα antagonists to determine whether this treatment can help to predict the outcome of IBD patients during maintenance therapy. Sixty patients with IBD were treated with TNFα antagonists and had their fecal calprotectin measured. Fecal calprotectin was found to be normalized (≤100 µg/g) in 31 patients and elevated in 29 patients. After 12 months, 26 of the 31 patients with normal fecal calprotectin levels were in clinical remission whereas only 11 of the 29 with elevated fecal calprotectin were in remission. A cutoff concentration of 139 µg/g was found to have a sensitivity of 72% and specificity of 80% to predict a risk of clinically active disease after one year (Molander et al., 2012)..

Molander et al. (2015) also studied whether fecal calprotectin can predict relapse after stopping TNFα-blocking therapy in IBD patients in remission. Forty-nine patients were examined, of which 15 relapsed (34 in remission). Relapsing patients showed an elevated fecal calprotectin for a median of 94 days before relapsing. Normal fecal calprotectin levels were “highly predictive” of clinical and endoscopic remission. The authors suggested that fecal calprotectin may be used as “a surrogate marker for predicting and identifying patients requiring close follow-up in clinical practice” (Molander et al., 2015).

Mao et al. (2012) performed a meta-analysis of the predictive capacity of fecal calprotectin in IBD relapse. A total of 672 patients (318 with ulcerative colitis, 354 with Crohn’s Disease) from six studies were examined. The authors found the pooled sensitivity and specificity of fecal calprotectin to predict relapse of quiescent IBD to be 78 and 73%, respectively. The area under the summary receiver-operating characteristic (sROC) curve was 0.83, and the diagnostic odds ratio was 10.31. The authors concluded that “as a simple and noninvasive marker, FC [fecal calprotectin] is useful to predict relapse in quiescent IBD patients” (Mao et al., 2012).

Rosenfeld et al. (2016) published a study to evaluate the perspective of gastroenterologists regarding the impact of fecal calprotectin on the management of patients with IBD. A total of 279 completed surveys were collected. Ninety surveys indicated fecal calprotectin testing was used to differentiate IBD from IBS, 85 indicated that fecal calprotectin was used to differentiate IBS symptoms from IBD in IBD patients, and 104 indicated fecal calprotectin was used as a marker for objective inflammation. Fecal calprotectin levels also resulted in a management change in 143 surveys, including 118 fewer colonoscopies. Overall, 272 surveys stated they would order fecal calprotectin again.

Abej et al. (2016) investigated the association between fecal calprotectin and other measures of clinical activity for patients with IBD. A total of 240 patients with IBD contributed 183 fecal samples, and a fecal calprotectin measurement above ≥250 µg was considered a positive result. Fecal calprotectin was associated with “colonoscopy findings of active IBD, low albumin, anemia, and elevated CRP.” The authors concluded that fecal calprotectin “is a useful marker of disease activity and a valuable tool in managing persons with IBD in clinical practice” (Abej et al., 2016).

Tham et al. (2018) showed that fecal calprotectin is an accurate surrogate marker of postoperative endoscopic recurrence of Crohn's disease. They evaluated the diagnostic sensitivity, specificity, and diagnostic odds ratio (DOR), and constructed summary receiver operating characteristic (SROC) curves in a meta-analysis of 54 studies; Nine studies were eligible for analysis. Diagnostic accuracy was calculated for fecal calprotectin values of 50, 100, 150 and 200 µg/g. A significant threshold effect was observed for all fecal calprotectin values. The optimal diagnostic accuracy was obtained for a fecal calprotectin value of 150 µg/g, with a pooled sensitivity of 70% [95% confidence interval (CI) 59-81%], specificity 69% (95% CI 61-77%), and DOR 5.92 (95% CI 2.61-12.17); the area under the SROC curve was 0.73 (Tham et al., 2018).

The cost-effectiveness of the use of fecal calprotectin in the diagnosis of IBD has been investigated (Yang et al., 2014). The authors compared cost-effectiveness of measuring fecal calprotectin before endoscopy compared to direct endoscopic evaluation alone. Fecal calprotectin screening was found to save $417 per adult patient, but delayed 2.2/32 adult diagnoses (of IBD) and 4.8/61. The authors noted that if endoscopic biopsy remained the diagnostic standard, direct endoscopic evaluation would cost an additional $18955 in adults to avoid one false-negative result from fecal calprotectin screening (Yang et al., 2014).

In a cross-sectional study, Campbell et al. (2021) assessed the clinical performance of the LIAISON Calprotectin Assay in differentiating inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS) against the Genova Diagnostics PhiCal test. 240 patients were included in the study in which 102 patients had IBD, 67 had IBS, and 71 had other GI disorders. Median fecal calprotectin levels were higher in IBD patients (522 µg/g) compared to IBS patients (34.5 µg/g). The LIAISON assay showed good correlation with the PhiCal test, holding a positive percent agreement of 97.8% and a negative percent agreement of 94.4%. Overall, the LIAISON Calprotectin Assay is efficient with a time to the first result of 35 minutes and "is a sensitive marker for distinguishing IBD from IBS with a cutoff of ~100 µg/g" (Campbell et al., 2021).

V. Guidelines and Recommendations

National Institute for Health and Care Excellence (NICE)

NICE published guidance on fecal calprotectin testing which included the following recommendations:

- "Fecal calprotectin testing is recommended as an option to support clinicians with the differential diagnosis of inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) in adults with recent onset lower gastrointestinal symptoms for whom specialist assessment is being considered, if cancer is not suspected and appropriate quality assurance processes and locally agreed care pathways are in place for the testing" (NICE, 2017).
- "Fecal calprotectin testing is recommended as an option to support clinicians with the differential diagnosis of IBD or non-IBD (including IBS) in children with suspected IBD who have been referred for specialist assessment (2017b)."

American Gastrointestinal Association (AGA)

Fecal Calprotectin Testing, continued

The AGA published a practice update on functional gastrointestinal symptoms in patients with IBD. The following best practice advice recommendations on fecal calprotectin were given regarding the diagnosis and management of functional gastrointestinal symptoms in patients IBD:

- "Best practice advice 1: A stepwise approach to rule-out ongoing inflammatory activity should be followed in IBD patients with persistent GI symptoms (measurement of fecal calprotectin, endoscopy with biopsy, cross-sectional imaging).
- Best practice advice 2: In those patients with indeterminate fecal calprotectin levels and mild symptoms, clinicians may consider serial calprotectin monitoring to facilitate anticipatory management (Colombel et al., 2019)."

American College of Gastroenterology (ACG)

The ACG Clinical Guideline (Lichtenstein et al., 2018) for the Management of Crohn's disease in adults recommends:

"Fecal calprotectin is a helpful test that should be considered to help differentiate the presence of IBD from irritable bowel syndrome (IBS) (strong recommendation, moderate level of evidence)."

"In patients who have symptoms of active Crohn's disease, stool testing should be performed to include fecal pathogens, *Clostridium difficile* testing, and may include studies that identify gut inflammation such as a fecal calprotectin."

"Fecal calprotectin and fecal lactoferrin measurements may have an adjunctive role in monitoring disease activity. Fecal markers may have a role in noninvasively monitoring disease activity in CD [Crohn's disease]. Studies have shown that both fecal lactoferrin and fecal calprotectin are sensitive markers of disease activity and correlate with a number of the endoscopic activity indices such as the colonic SES-CD. There have been several studies that suggest that levels of fecal calprotectin can be used to monitor patients for postoperative recurrence after ileocolic resection for Crohn's disease. Levels of >100 µ g/g indicate endoscopic recurrence with a sensitivity in the range of 89%. In patients with an infliximab-induced remission, fecal calprotectin of >160 µ g/g has a sensitivity of 91.7% and a specificity of 82.9% to predict relapse… The presence of biomarkers of disease activity can be assessed (such as CRP, fecal calprotectin) but should not exclusively serve as end point for treatment as normalization of the biomarker can occur despite having active mucosal inflammation/ulceration… Although not specific for CD activity, determination of serum CRP and/or fecal calprotectin is suggested as a useful laboratory correlate with disease activity assessed by the CDAI (Lichtenstein et al., 2018)."

The Crohn's Disease Activity Index (CDAI) is a tool that can provide a numerical value in assessing Crohn's disease; however, fecal calprotectin is not a criterion of the index. Within the supplemental information of the guidelines, the authors state, "This is a weighted subjective tool that includes scores for liquid bowel movements per day, general wellbeing, abdominal pain and extra-intestinal manifestations. This index does require 7 days of measurements making it difficult to use in the clinic setting. Due to the subjective nature of some of the measurements it is not an optimal tool for measuring disease activity and is generally not used in routine clinical practice"(Lichtenstein et al., 2018).

The guidelines do not address the frequency of fecal calprotectin testing for adjunctive monitoring.

The ACG also published guidelines for clinical management of ulcerative colitis in adults in 2019. In it, they note that "Fecal calprotectin (FC) can be used in patients with UC as a noninvasive marker of disease activity and to assess response to therapy and relapse" (Rubin et al., 2019).

The ACG also recommends:

- "Stool testing to rule out *Clostridioides difficile (C. diff)* in patients suspected of having UC (strong recommendation, very low quality of evidence)."
- Recommends against "serologic antibody testing to establish or rule out a diagnosis of UC (strong recommendation, very low quality of evidence)."
- Recommends against serologic antibody testing to determine the prognosis of UC (strong recommendation, very low quality of evidence)" (Rubin et al., 2019).

In 2021, the ACG published guidelines on the management of irritable bowel syndrome. They recommend that that fecal calprotectin, either fecal calprotectin 1 or fecal lactoferrin 2 and C-reactive protein 1, be checked in patients with suspected IBS and diarrhea symptoms to rule out inflammatory bowel disease. ACG includes that two fecal-derived markers of intestinal inflammation, fecal lactoferrin (FL) and fecal calprotectin (fCal), are both diagnostically useful and could be superior to serologic tests such as CRP or ESR regarding discriminating IBD from IBS. "In summary, fCal and FL are safe, noninvasive, generally available, and can identify IBD with good accuracy" (Lacy et al., 2021).

European Crohn's and Colitis Organisation (ECCO)

The ECCO released a consensus on diagnosis and management of ulcerative colitis (UC). In it, they state that fecal calprotectin should be included on an initial investigation of UC. ECCO considers fecal calprotectin an "accurate" marker of colonic inflammation and "a useful non-invasive marker in the follow-up of UC patients" (Magro et al., 2017).

The ECCO also provided a statement on diagnosis and management of Crohn's Disease. ECCO notes that fecal calprotectin may be used in the initial laboratory investigation. Fecal calprotectin is also observed to be an emerging surrogate marker for mucosal healing, but has not demonstrated a clear predictive value. Fecal calprotectin may also help in monitoring disease activity (Gomollón et al., 2016).

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

The ECCO-ESGAR published guidelines for the diagnostic assessment in IBD. When monitoring known IBD cases, the following guidelines were provided:

- "Response to treatment in active ulcerative colitis [UC] should be determined by a combination of clinical parameters, endoscopy, and laboratory markers such as C-reactive protein [CRP] and faecal calprotectin [EL1]
- In patients with UC who clinically respond to medical therapy, mucosal healing [MH] should be determined endoscopically or by faecal calprotectin [FC] approximately 3 to 6 months after treatment initiation [EL5] (Maaser et al., 2019)"

Fecal Calprotectin Testing, continued

A relevant portion of "**Table 1.** Markers of disease activity for monitoring asymptomatic IBD patients" is shown below (Maaser et al., 2019):

	Validity (correlation with gold standard)	**Responsiveness to changes in condition**	**Signal-to-noise ratio (ability to differentiate changes in condition from background variability)**	**Practicality**
Endoscopy	Gold standard	Gold standard	Gold standard	Low
Faecal calprotectin	Good	Good Rises quickly in case of relapse; falls rapidly with successful treatment	Moderate Risk of false-positive results	High Possible reluctance of patients for repeated stool collection

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

In March 2006, the PhiCal™ (Genova Diagnostics) quantitative ELISA test for measuring concentrations of fecal calprotectin in fecal stool was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) processes. This test is indicated to aid in the diagnosis of inflammatory bowel disease (IBD) and to differentiate IBD from irritable bowel syndrome (IBS); it is intended to be used in conjunction with other diagnostic testing and clinical considerations (FDA, 2006). On December 26, 2018, a successor device called "LIAISON Calprotectin, LIAISON Calprotectin Control Set, LIAISON Calprotectin Calibration Verifiers, LIAISON Q.S.E.T. Buffer, LIAISON Q.S.E.T. Device" was approved. The new description is as follows: "The DiaSorin LIAISON® Calprotectin assay is an in vitro diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON® Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, and as an aid in differentiation of IBD from irritable bowel syndrome (IBS). Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures. The test has to be performed on the LIAISON® XL Analyzer" (FDA, 2018).

In January 2014, CalPrest® (Eurospital SpA, Trieste, Italy) was cleared for marketing by FDA through the 510(k) processes. According to the FDA summary, CalPrest® "is identical" to the PhiCal™ test "in that they are manufactured by Eurospital S.p.A. Trieste, Italy. The only differences are the name of the test on the labels, the number of calibrators in the kit and the dynamic range of the assay." CalPrest®NG (Eurospital SpA) was cleared for marketing in November 2016 (FDA, 2016).

On October 16, 2018, the FDA approved the QUANTA Flash Calprotectin And Fecal Extraction Device. The device's intended use is as follows: "QUANTA Flash Calprotectin is a chemiluminescent immunoassay for the quantitative determination of fecal calprotectin in extracted human stool samples. Elevated levels of fecal calprotectin, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease), and in the differentiation of IBD from irritable bowel syndrome (IBS)." This device has a predicate device, which was approved in 2017 (FDA, 2018a).

On December 26, 2018, the FDA approved the LIAISON Calprotectin Assay. The device's intended use is as follows: "The DiaSorin LIAISON® Calprotectin assay is an in vitro diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON® Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, and as an aid in differentiation of IBD from irritable bowel syndrome (IBS). Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures" (FDA, 2018b).

On September 24, 2019, BUHLMANN Laboratories AG received FDA approval for the Buhlmann FCAL Turbo And CALEX Cap fecal calprotectin extraction device. This device is to be used in conjunction with the automated calprotectin test, BÜHLMANN fCAL® turbo. The BÜHLMANN fCAL® turbo is an in vitro diagnostic assay which quantitatively measures fecal calprotectin (FDA, 2019).

Rapid fecal calprotectin tests, such as CalproSmart™, are available internationally for use as point-of-care testing, but these have not been approved for use in the U.S. by the 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 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
83993	Assay for calprotectin fecal

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

G2061 Fecal Calprotectin Testing

Flow Cytometry

Policy #: AHS – F2019	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 4/28/22, 8/19/22 (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

Flow cytometry is a technique for live cell analysis that measures optical light scattering features to determine physical characteristics (Adan et al., 2017). This instrument is beneficial for calculating the number of cells in a biologic sample, as well as for measuring cellular properties, such as size, shape, viability, and granularity (Verbsky & Routes, 2022). Flow cytometry may also be used for diagnostic and prognostic purposes when monitoring certain diseases, and for identifying the presence of specific biomarkers.

Flow cytometry-derived DNA content can be used for cell cycle analysis to estimate the percentages of a cell population in the various phases of the cell cycle; it can also be used with other reagents to analyze only the S phase. A S-phase fraction (SPF) is an assessment of how many cells are actively synthesizing DNA (UIHC, 2016). It is used as a measure of cell proliferation, particularly for cancer (Pinto et al., 1999). A high SPF value is indicative of rapid cancer growth (ACS, 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.

Flow Cytometry, continued

1. Flow cytometry immunophenotyping of cell surface markers **MEETS COVERAGE CRITERIA** for any of the following conditions:
    a. For individuals with cytopenias, lymphomas, leukemia and lymphoproliferative disorders or myelodysplastic syndrome;
    b. For B-cell monitoring for immunosuppressive disorders;
    c. For T-cell monitoring for HIV infection and AIDS
    d. For individuals with mast cell neoplasms
    e. For individuals with paroxysmal nocturnal hemoglobinuria
    f. For post-operative monitoring of members who have undergone organ transplantation
    g. For individuals with plasma cell disorders
    h. For individuals with primary Immunodeficiencies (PIDs), and PIDs involving T, NK
    i. Minimal Residual Disease (MRD)
    j. For individuals with primary Platelet Disorders (non-neoplastic)
    k. For individuals with red cell and white cell disorders(non-neoplastic)
2. The following reimbursement limitations will apply for flow cytometry:
    a. For flow cytometric immunophenotyping for the assessment of potential hematolymphoid neoplasia, use codes 88184-88189.
    b. Code 88184 should be used for the first marker, per specimen, and is reimbursable up to a maximum of two units per date of service.
    c. Code 88185 should be used for each additional marker and is reimbursable up to a maximum of 35 units, per date of service.
    d. In patients with a neoplasm with an established immunophenotype, subsequent tests for that neoplasm should be limited to diagnostically relevant markers.
    e. Codes 88187, 88188, and 88189 should not be used together for a single specimen in any combination.
    f. Codes 88187, 88188, and 88189 are reimbursed at one unit per specimen, up to two specimens, per date of service.
    g. Codes 88187-88189 should not be used in conjunction with codes 86355, 86356, 86357, 86359, 86360, 86361, 86367.
    h. Use codes 86355, 86357, 86359, 86360, 86361, or 86367 for cell enumeration. These codes are reimbursable as single units only.

Flow Cytometry, continued

3. Coding:

Bill Type Codes

012x	Hospital Inpatient (Medicare Part B only)
013x	Hospital Outpatient
014x	Hospital - Laboratory Services Provided to Non-patients
018x	Hospital - Swing Beds
021x	Skilled Nursing - Inpatient (Including Medicare Part A)
022x	Skilled Nursing - Inpatient (Medicare Part B only)
023x	Skilled Nursing - Outpatient
071x	Clinic - Rural Health
077x	Clinic - Federally Qualified Health Center (FQHC)
085x	Critical Access Hospital

Group 1 Codes:

88182	Cell marker study
88184	Flowcytometry/ tc 1 marker
88185	Flowcytometry/tc add-on
88187	Flowcytometry/read 2-8
88188	Flowcytometry/read 9-15
88189	Flowcytometry/read 16 & >

Group 2: Quantitative Codes in immunology section

Group 2 Codes:

86355	B cells total count
86356	Mononuclear cell antigen
86357	Nk cells total count
86359	T cells total count
86360	T cell absolute count/ratio
86361	T cell absolute count
86367	Stem cells total count

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) Measurement of flow cytometry-derived DNA content (DNA Index) or cell proliferative activity (S-phase fraction or % S-phase) for prognostic or therapeutic purposes in the routine clinical management of cancers **DOES NOT MEET COVERAGE CRITERIA.**

III. Scientific Background

Flow cytometry is a laboratory technique with the capability to measure optical and fluorescence characteristics from single cells or other particles between 0.2 and 150 micrometers in size, such as microorganisms, nuclei or chromosome preparations suspended in fluid (Brown & Wittwer, 2000; Verbsky & Routes, 2022). More than 100 companies constitute the flow cytometry market, leading to an industry worth of more than $3 billion (Robinson & Roederer, 2015).

A typical flow cytometer contains five main components: a flow cell, a laser, optical parts, detectors which amplify signals, and an electronic or computer system (Verbsky & Routes, 2022). This device measures thousands of cells instantaneously by passing them through the laser beam, and it can even sort the cells into 96- or 384-well plates, tubes, and slides based on identified cellular properties (McKinnon, 2018). Size is determined by the forward angle light scatter, and internal properties such as cellular granularity are measured by the right-angle light scatter (Brown & Wittwer, 2000; Verbsky & Routes, 2022). These fluorescent light signals are converted into electronic signals and then analyzed by a computer to generate final results (McKinnon, 2018).

Fluorescent reagents may be used to enhance a sample before administration into the flow cytometer. These reagents may include DNA binding dyes, fluorescently conjugated antibodies, viability dyes, fluorescent expression proteins, and ion indicator dyes (McKinnon, 2018). Each fluorescent dye binds to cellular components differently, leading to distinguished outcomes when passed by the light source. A fluorochrome, or chemical that can re-emit light when excited, can assist in the detection of specific cellular properties. The use of multiple fluorochromes at once allows several characteristics to be identified instantaneously as different colors emit different wavelengths of light; common dyes include propidium iodide, phycoerythrin, and fluorescein (Brown & Wittwer, 2000).

Immunophenotyping is the most common use of flow cytometry and entails the identification of cellular markers from the immune system, such as T cell subsets and cytokines, as well as antigen-specific responses. Unfortunately, immunophenotyping faces issues in the clinical world due to a lack of standardized procedures (Finak et al., 2016). Current instruments allow for up to 28 colors to be used in immunophenotyping experiments, yet many researchers use less than this (McKinnon, 2018).

Flow cytometry as a laboratory technique can measure and assess DNA ploidy through cell cycle analysis. DNA synthesis and replication errors are associated with cancer. Cancer is the uncontrolled growth and spread of abnormal cells and is increasingly shown to be initiated, propagated, and maintained by somatic genetic events (Johnson et al., 2014).

During the cell cycle, DNA synthesis is tightly regulated and only performed just as the cell is about to divide. This step of DNA replication is called the “S-phase” (Christensen, 2021). Dysfunction of DNA replication is significantly associated with cancer, and cancers frequently involve damage or removal of molecular regulators of replication (Van der Aa et al., 2013). Assessment of the fraction of cells in S-phase has been proposed as an indicator of neoplasm aggression. S-phase fraction (SPF) is thought to reflect proliferative activity of cancer and may provide prognostic or therapeutic information (Ermiah et al., 2012). Elevated proliferative activity may predict a worsened disease-free or overall survival in several cancers, such as breast, non-small cell lung, colorectal, ovarian, kidney, bladder, prostate, and

Flow Cytometry, continued

endometrial cancers (Bagwell et al., 2001; Gawrychowski et al., 2003; Kenney et al., 2008; Mangili et al., 2008; Pinto et al., 2011; Ross, 1996). However, data supporting the use of SPF as a prognostic tool appears to be inconsistent at best (Locker et al., 2006).

Clinical Utility and Validity

Technically, any biologic sample can be analyzed by flow cytometry. However, blood is the most common sample type, including both whole blood and peripheral blood mononuclear cells (Verbsky & Routes, 2022). Flow cytometry can be employed for prognostic and diagnostic purposes. This technique has been used to identify both primary immunodeficiencies and secondary or acquired immunodeficiencies such as HIV (Verbsky & Routes, 2022). Primary immunodeficiencies represent more than 300 known genetic disorders, and flow cytometry is a major component of the diagnosis of these disorders (Abraham & Aubert, 2016). Flow cytometry may also be used for prenatal diagnoses, hematology, transplantation, crop improvement, sperm sorting for sex preselection, post-bone marrow transplantation analyses, and during immunosuppression and chemotherapy treatments (Halder et al., 2017; Verbsky & Routes, 2022).

Today, many assays have been developed for flow cytometry purposes. These assays can identify biomarkers for cancer and stem cells, DNA and RNA, reactive oxygen species, and the functional status of yeast or bacteria (Robinson & Roederer, 2015). Newer techniques have also been developed such as mass cytometry: the combination of flow cytometry and mass spectrometry (Cosma et al., 2017). Flow fluorescent in situ hybridization (FISH) is another combinatory technique which is the combination of fluorescent in situ hybridization in suspension (FISHIS) and flow cytometry using DNA or gene-specific probes.

Flow cytometry techniques have been used to identify several types of cancer. Fromm et al. (2009) used flow cytometry to identify classical Hodgkin lymphoma, neoplastic Hodgkin, and Reed Sternberg cells in lymph nodes with 88.7% sensitivity and 100% specificity. Paiva, Merino, and San Miguel (2016) state that next generation multiparameter flow cytometry "should be considered mandatory in the routine evaluation of multiple myeloma patients both at diagnosis, and after therapy, and represents an attractive technique to integrate with high-throughput DNA and RNA-seq methods to help in understanding the mechanisms behind dissemination and chemoresistance of multiple myeloma." Finally, Novikov et al. (2019) used flow cytometry immunophenotyping to identify malignant T-cell clones in mature peripheral T-cell lymphomas with 97% sensitivity and 91% specificity.

Wang et al. (2019) published a study on the applicability of multiparameter (multicolor) flow cytometry (MFC) for detecting MRD to predict relapse in patients with AML after allogeneic transplantation. The researchers also compared MFC to MRD status determined using real-time quantitative polymerase chain reaction (RT-qPCR) from 158 bone marrow samples from 44 different individuals. "Strong concordance was found between MFC-based and RT-qPCR-based MRD status ($\kappa = 0.868$)." Moreover, for individuals in complete remission (CR), "the positive MRD status detected using MFC was correlated with a worse prognosis [HRs (*P* values) for relapse, event-free survival, and overall survival: 4.83 (<0.001), 2.23 (0.003), and 1.79 (0.049), respectively]; the prognosis was similar to patients with an active disease before HSCT [hematopoietic stem cell transplantation] (Wang et al., 2019)."

Flow Cytometry, continued

Clinical Utility and Validity of DNA Ploidy Cell Cycle Analysis

Carloni et al. (2017) evaluated the associations between SPF and peritoneal carcinomatosis from ovarian cancer. Fifty-three patients were examined, and although SPF differed among the different ploidy categories, no significant correlation was found between SPF and clinical pathological characteristics of patients. However, the authors did find that sensitivity to taxol was correlated with SPF, therefore concluding that "ploidy and SPF could facilitate the choice of therapy for patients with peritoneal carcinomatosis" (Carloni et al., 2017).

Svanvik et al. (2019) examined 1113 patients diagnosed with stage I-III grade 1-3 endometrioid endometrial carcinoma in 2006-2011. They evaluated both DNA ploidy and SPF and set the SPF cutoff at 8%. The authors found that 5-year relative survival was significantly associated with SPF and DNA ploidy through a univariate statistical analysis. However, when other variables such as age, grade, and stage were added, SPF and DNA ploidy became statistically insignificant. Therefore, the authors concluded that "S-phase fraction, DNA ploidy, and p53 overexpression did not improve identification of high-risk patients by stage, grade, and age in stage I-III endometrioid endometrial carcinoma" (Svanvik et al., 2019).

Thomas et al. (2020) completed a study to analyze the prognostic implications of DNA repair, DNA ploidy and telomerase in the malignant transformation risk assessment of leukoplakia. Samples from 200 patients with oral leukoplakia, 100 patients with oral cancer and 100 healthy controls were analyzed. The DNA ploidy content was measured with high resolution flow cytometry; the authors identified that "There was significant difference in the distribution of ploidy status, telomerase activity and DNA repair capacity among control, leukoplakia and oral cancer group ($p<0.001$). When the molecular markers were compared with histological grading of leukoplakia, both DNA ploidy analysis and telomerase activity showed statistical significance ($p<0.001$)" (Thomas et al., 2020).

Taniguchi et al. investigated the correlation between flow cytometry parameters such as DNA ploidy, DNA index and S-phase fraction and clinical prognostic factors such as mitotic count and Ki-67 labelling index (LI). The cancer of interest was "gastrointestinal stromal tumours (GIST)" and eighteen specimens from laparoscopic local gastrectomy were analyzed. The authors found these flow cytometry parameters to correlate well with mitotic count ≤5 and Ki-67 LI ≤6. DNA index was found to be 83.3% accurate in predicting mitotic count ≤5 and 77.8% accurate in predicting Ki-67 LI ≤6, while S-phase fraction was found to be 94.4% accurate and 88.9% accurate, respectively. The authors concluded that "Rapid flow cytometry parameters can classify risk without the need for histological analysis" (Taniguchi et al., 2021).

Panwar et al. (2021) studied the evaluation of DNA ploidy and S-phase fraction in fine needle aspirates from breast carcinoma. Fifty breast cancer patients who underwent fine needle aspiration cytology (FNAC) were included in the study. The samples from FNAC underwent DNA ploidy and SPF analysis and Ki-67 was estimated. SPF and Ki-67 were compared with each other. "On DNA flow cytometry, 27 (54%)

cases were aneuploid and 23 (46%) cases were diploid. The median SPF was 12.43% and 4.03% in aneuploid and diploid tumors respectively. Median Ki-67 among aneuploid tumors was 28.6% compared to 8.7% among diploid tumors. Aneuploid tumors were significantly associated with higher values of SPF and Ki-67, with Kappa 0.437 and agreement of 72%. Diploid tumors showed lower values of SPF and Ki-67, with Kappa 0.455 and agreement of 72.7%. Correlation among SPF and Ki-67 was highly significant with Kappa value 0.446, P value of .002 and agreement of 72.3%" (Panwar et al., 2021). The authors conclude that DNA ploidy and proliferative activity by flow cytometric SPF estimation can provide valuable prognostic information in breast cancer diagnosis.

IV. Guidelines and Recommendations

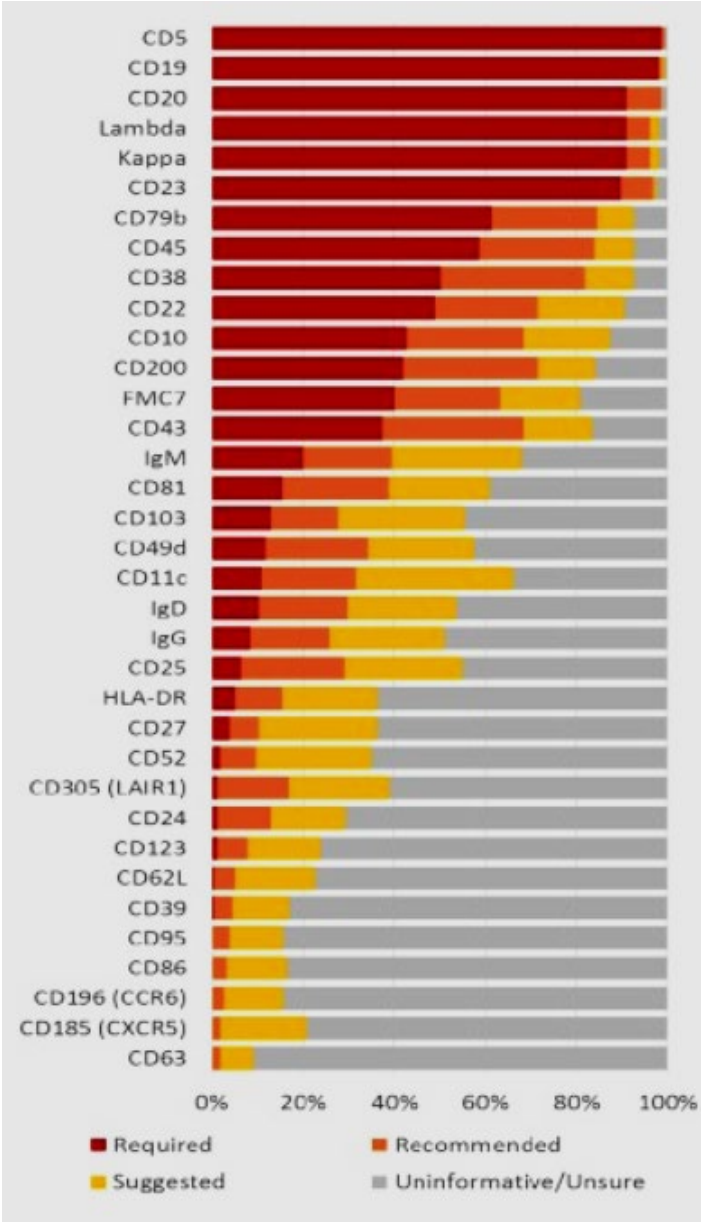
Flow cytometry is broadly used for many conditions such as cancers, which are mentioned across many different societies. The below section is not a comprehensive list of guidance for flow cytometry.

The European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation Project

This group has published guidelines on chronic lymphocytic leukemia (CLL) in hopes to determine "35 potential flow cytometry markers as being "required," "recommended," "suggested," "uninformative," or "not sure" for the diagnosis of CLL (Rawstron et al., 2018)." A marker is required if >75% of ERIC/ESCCA members determine that it should be required, and a marker is pushed forward for review if >50% of all members determine that it should be recommended or required. Results are shown in the following figure:

Figure 1 [taken from (Rawstron et al., 2018)]:

Flow Cytometry, continued



Flow Cytometry, continued

International/European Leukemia Net Working Group for Flow Cytometry in Myelodysplastic Syndromes

An international working party was organized to develop flow cytometry techniques in the classification of myelodysplastic syndromes (MDS). The group has stated the following guidelines:

- "In laboratories where comprehensive immunophenotyping can be performed, an MDS immunophenotyping panel… is recommended.
- In patients with cytological findings suggesting MDS of RCUD (refractory anemia subtype) or refractory anemia with ringed sideroblasts categories, aberrant flow cytometry (FCM) findings in the granulopoietic or myelomonocytic lineages may indicate multilineage dysplasia, which is of prognostic significance. Morphological findings in these cases should be thoroughly re-evaluated to avoid misclassification.
- It is important to note even small populations of myeloid progenitors with multiple immunophenotypic aberrant features (such as aberrant expression of CD7, CD56 or CD11b, see Table 1), since they indicate a higher risk of progression to AML. FCM findings in these cases should be included in the individual risk assessment (Porwit et al., 2014)."

The Clinical Cytometry Society 2006 Bethesda International Consensus

In 2006, a panel of subject matter experts convened to define the clinical indications that warrant the use of flow cytometry, as well as to identity of the reagents that should be used in the initial and secondary evaluations for those conditions (Davis et al., 2007). The output of that gathering was the 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry. The panel indicated that flow cytometry is useful for the evaluation of cytopenias, elevated leukocyte count, observation of atypical cells or blasts and evaluation of body fluids, plasmacytosis or monoclonal gammopathy, organomegaly and tissue masses, and certain patient monitoring indications.

The Bethesda recommendations indicate that flow cytometry is not indicated for mature neutrophilia, polyclonal hypergammaglobulinemia, polycythemia, thrombocytosis, and basophilia because "they are usually not associated with hematolymphoid malignancy or associated with hematolymphoid neoplasms that are not detectable by" flow cytometry.

The Bethesda recommendations also indicate that selection of reagents for the initial evaluation panel should be based on specimen type (peripheral blood, bone marrow, tissue, etc.), clinical information and cell morphology studies. They identify initial panels for specific indications that range from a total of 4 reagents to a maximum of 12 reagents.

For secondary evaluation, where the initial evaluation is not conclusive or informative, the Bethesda recommendations again identify groups of reagents that should be used, based on indication. The secondary panels ranged from 5 to 23 reagents.

Flow Cytometry, continued

Specific recommendations for the initial evaluation were:

- B cells: CD5, CD10, CD19, CD20, CD45, Kappa, Lambda
- T cells and NK cells: CD2, CD3, CD4, CD5, CD7, CD8, CD45, CD56
- Myelomonocytic cells: CD7, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD45, CD56, CD117, HLA-DR
- Myelomonocytic cells (limited): CD13, CD33, CD34, CD45
- Plasma cells CD19, CD38, CD45, CD56

For secondary evaluation, the Bethesda recommendations were:

- B cells: CD9, CD11c, CD15, CD22, cCD22, CD23, CD25, CD13, CD33, CD34, CD38, CD43, CD58, cCD79a, CD79b, CD103, FMC7, Bcl-2, cKappa, cLambda, TdT, Zap-70, cIgM
- T cells and natural (NK) cells: CD1a, cCD3, CD10, CD16, CD25, CD26, CD30, CD34, CD45RA, CD45RO, CD57, ab-TCR, gd-TCR, cTIA-1, T-beta chain isoforms, TdT
- Myelomonocytic cells: CD2, CD4, CD25, CD36, CD38, CD41, CD61, cCD61, CD64, CD71, cMPO, CD123, CD163, CD235a
- Plasma cells: CD10, CD117, CD138, cKappa, cLambda

The American Society of Clinical Oncology Tumor Markers Expert Panel (ASCO)

In 2006, the ASCO updated the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of gastrointestinal cancers. These recommendations state that "Neither flow-cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should be used to determine prognosis of early-stage colorectal cancer (Locker et al., 2006)."

In 2007, the ASCO updated the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast cancer (Harris et al., 2007); the authors noted that "DNA/ploidy by flow cytometry demonstrated insufficient evidence to support routine use in clinical practice."

College of American Pathologists and the American Society of Hematology

In 2016, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) published a joint guideline to outline their recommendations for the initial diagnostic workup of acute leukemia. Among their 27 recommendations, three statements (each rated "Strong Recommendation") explicitly address the leveraging of flow cytometry in said process:

"5. In addition to morphologic assessment (blood and bone marrow), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (i.e., karyotype), appropriate molecular genetic and/or fluorescent in situ hybridization (FISH) testing, and flow cytometric immunophenotyping (FCI). The flow cytometry panel should be sufficient to distinguish acute myeloid

leukemia (including acute promyelocytic leukemia), T-cell acute lymphoblastic leukemia (T-ALL) (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and acute leukemia of ambiguous lineage on all patients diagnosed with acute leukemia. FISH and/or molecular genetic testing does not, however, replace conventional cytogenetic analysis.

Note — If sufficient bone marrow aspirate or peripheral blood material is not available for FCI, immunohistochemical studies may be used as an alternative method for performing limited immunophenotyping. In addition, a second bone marrow core biopsy can be obtained and submitted, unfixed in tissue culture media, for disaggregation for genetic studies and flow cytometry."

"10. For patients with suspected or confirmed acute leukemia, the pathologist may use flow cytometry for the evaluation of CSF."

"12. For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease."

A final recommendation (also a "Strong Recommendation") mentioning flow cytometry referred to the use of its data, such that

"24. If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded when they become available."

International Society on Thrombosis and Haemostasis (ISTH)

The International Society on Thrombosis and Haemostasis SSC Subcommittee outlined several recommendations for which flow cytometric analysis of inherited and acquired platelet disorders should occur. Those clinical settings in which it believed would be helpful, guided by expert consensus, are reported below:

"Diagnosis of inherited or acquired deficiencies of platelet surface glycoproteins (BSS, GT, inherited or immune-mediated GPVI defects)

Diagnosis of platelet alpha granule secretion defects (such as gray platelet syndrome)

Diagnosis of defects in specific platelet activation (signaling) pathways (such as RASGRP2, P2Y12, or TXA2R disorders)

Diagnosis of GFI1B macrothrombocytopenia associated to platelet expression of CD34

Diagnosis of disorders of platelet procoagulant activity (such as Scott syndrome and Stormorken syndrome)

Assessment of increased platelet activation in prothrombotic syndromes (diabetes, anti-phospholipid syndrome or secondary to drug induced, non-immune platelet activation)

Monitoring, if applicable, pharmacodynamic effect of P2Y12 antagonists (ticlopidine, clopidogrel, prasugrel, ticagrelor, cangrelor) with specifically designed test such as VASP P2Y12

Determination of the fraction of immature platelets" (Frelinger et al., 2021).

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines on diagnosis and/or management of Breast Cancer (Version 2.2023), Cervical Cancer (Version 1.2023), Colon Cancer (Version 3.2022), Small Cell Lung Cancer (Version 3.2023), and Non-Small Cell Lung Cancer (Version 3.2023) do not mention cell proliferation activity (S-phase fraction or % S-phase) as a management tool (NCCN, 2023).

International Society of Gynecological Pathologists (ISGyP) Endometrial Cancer Project: Guidelines from the Special Techniques and Ancillary Studies Group

These guidelines focus on biomarkers and their potential use for endometrial carcinoma.

The guideline remarks that "Other than markers which are useful in diagnosis, there are few specific studies that provide definitive evidence for the routine use of IHC [immunohistochemistry] or ploidy analysis in determining the prognosis of EC" and that "There is some literature on the association of ploidy with prognosis, with promising results, but there is a lack of definitive studies to determine its true prognostic impact".

Overall, the guideline states that "Clearly, large prospective, well defined, uniform studies are needed to determine the possible role of IHC for specific biomarkers and ploidy analysis in the clinical setting." (Cho et al., 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 for clinical use.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86355	B cells, total count

Flow Cytometry, continued

86356	Mononuclear cell antigen, quantitative (e.g., flow cytometry), not otherwise specified, each antigen (Do not report 88187-88189 for interpretation of 86355, 86356, 86357, 86359, 86360, 86361, 86367)
86357	Natural killer (NK) cells, total count
86359	T cells; total count
86360	absolute CD4 and CD8 count, including ratio
86361	absolute CD4 count
86367	Stem cells (i.e., CD34), total count (For flow cytometric immunophenotyping for the assessment of potential hematolymphoid neoplasia, see 88184-88189)
88182	Flow Cytometry, cell cycle or DNA analysis
88184	Flow Cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker.
88185	Each additional marker (listed separately in addition to code 88184 for the first marker)
88187	Flow cytometry, interpretation; 2 to 8 markers
88188	9 to 15 markers

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 read as follows: "Cytopenias, lymphomas, leukemia, myeloproliferative and lymphoproliferative disorders, or myelodysplastic syndrome" and removed coverage criteria #1i, 1k, and 1j due to repetition; removed CPT code 88199.
8/19/22	Deleted previous coverage criteria #1j (Molar pregnancy), and modified coverage criteria #2e and #2f to ensure clarity.

Folate Testing

Policy #: AHS – G2154	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/23/22, 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

Folate, or vitamin B9, is a generic term for a water-soluble vitamin obtained from the diet that is involved in the transfer of methyl groups (i.e. single carbon-containing groups) in multiple biochemical metabolic pathways, including nucleic acid biosynthesis and methionine/homocysteine metabolism. Folate metabolism is closely linked to vitamin B12, cobalamin. Folate deficiency can be implicated in many disease states and processes; however, it is usually easily remedied with either a change in diet or a dietary supplement of the synthetic form, folic acid (Means Jr & Fairfield, 2023a; NIH, 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 diagnosed with megaloblastic or macrocytic or unexplained anemia and for whom the anemia and/or macrocytosis does not resolve after folic acid treatment, measurement of serum folate concentration **MEETS COVERAGE CRITERIA.**
2. For all indications not described above, measurement of serum folate concentration **DOES NOT MEET COVERAGE CRITERIA**.

3. For all indications, measurement of red blood cell (RBC) folate **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.

4. For all situations, folate receptor autoantibody testing **DOES NOT MEET COVERAGE CRITERIA.**

III. Scientific Background

Folate, or vitamin B9, naturally occurs as polyglutamated compounds (pteroylpolyglutamates) in many plant and animal products. The synthetic form is a monoglutamate-containing compound called folic acid. Folic acid is more chemically stable for commercial production and storage, but it is less bioavailable than the naturally occurring folate (Means Jr & Fairfield, 2023a). Biochemically, folate is a coenzyme in single-carbon transfers *in vivo* and is directly linked to the cobalamin (vitamin B12) cycle, methionine metabolism, and nucleic acid biosynthesis. Dietary folates are hydrolyzed via γ-glutamylhydrolase (or folate conjugase) prior to absorption in the intestinal mucosa (IOM, 1998). Both folate and vitamin B12 are required for formation of 5,10-methylene tetrahydrofolate, which is the cofactor involved in purine synthesis. Methylenetetrahydrofolate reductase (MTHFR) is the enzyme responsible in converting 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, which is required for methionine synthase, the enzyme that converts homocysteine to methionine. The interlinked one-carbon cycle is depicted in the figure below with the metabolites assayed in clinical laboratories in bold (Finer et al., 2013).

Folate Testing, continued

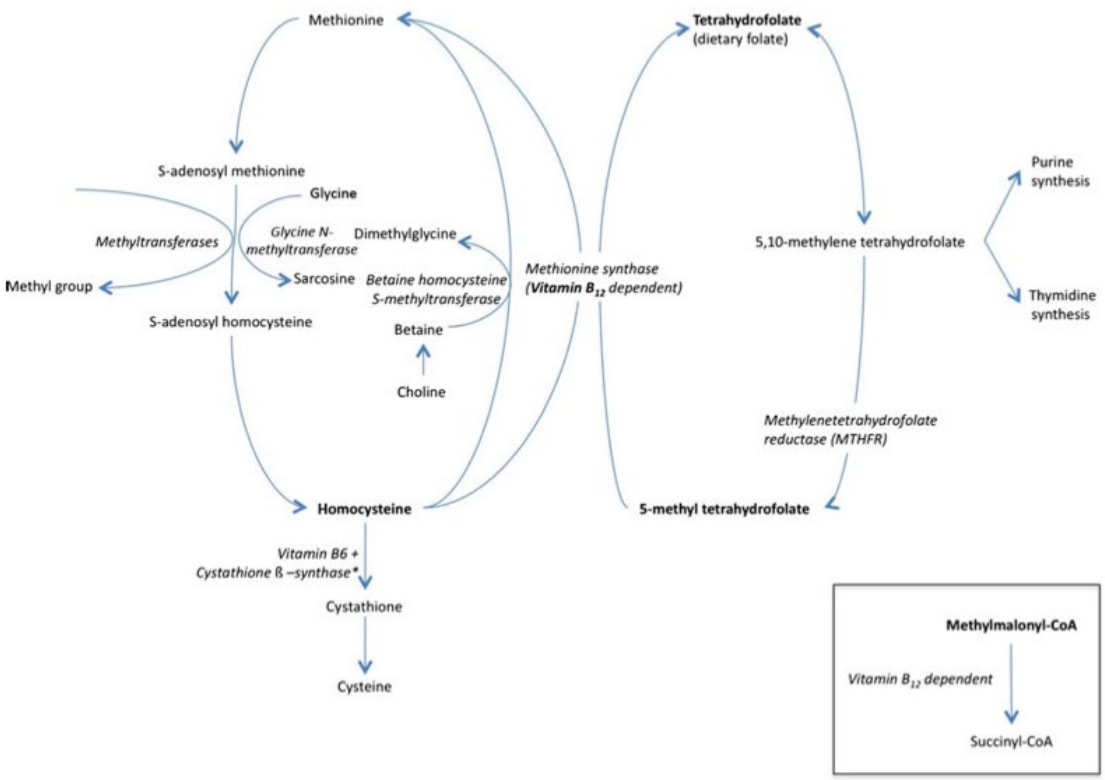

FIGURE 1 The one-carbon cycle. Metabolites readily assayed in clinical laboratories are highlighted in bold.

Role of Folate in Anemia

Anemia occurs when the body lacks healthy red blood cells (RBCs), leading to an insufficient amount of oxygen delivered to tissues. Typical symptoms of anemia include fatigue, weakness, pale skin and lightheadedness.

Macrocytic anemia refers to anemias that have high mean corpuscular volume with large RBCs. Mean corpuscular volume, or mean cell volume, can be defined as the average volume of RBCs in an individual. Megaloblastic anemia is a specific macrocytic anemia due to nucleic acid metabolic defects that result in "nuclear-cytoplasmic dyssynchrony, reduced number of cell divisions in the bone marrow, and nuclear abnormalities in both myeloid and erythroid precursors" caused by folate and/or vitamin B12 deficiency (Means Jr & Fairfield, 2023b). These abnormal RBCs are the principle clinical manifestations of folate deficiency and symptoms "include weakness, fatigue, difficulty concentrating, irritability, headache, heart palpitations, and shortness of breath" (NIH, 2018).

Folate Testing, continued

Folate and Neural Tube Defects (NTDs)

Neural tube defects (NTDs) develop early in pregnancy and are malformations of the brain and/or spine that include spina bifida and anencephaly. Folate deficiency is directly linked to NTDs. The role of folate in NTD development is not well-characterized. The role of folate in either the methylation cycle or nucleic acid synthesis has been suggested to play a part in NTD development during embryogenesis, and some studies have indicated that it is the bioavailability of specific folates in the pregnant individual that can increase the likelihood of NTDs (Imbard et al., 2013; Rothenberg et al., 2004). Individuals typically do not obtain enough folate from diet alone, so individuals of childbearing age are recommended to take a synthetic folic acid supplement to decrease the likelihood of NTDs in offspring (Bibbins-Domingo et al., 2017). To decrease the occurrence of NTDs and folate deficiency, the United States and Canada mandated folic acid supplementation to cereal grains in 1998, and as of March 2018 "92 countries have legislation to mandate fortification of at least one industrially milled cereal grain" (FFI, 2021).

It is notable that the prevalence of folate deficiency, and the prevalence of NTDs has declined in countries with routine folic acid supplementation (Crider et al., 2011). A review by Imbard et al. (2013) of 17 different studies on the impact of folic acid fortification of NTD rates show that 16 show a decrease in the rate of NTDs. Only one study of the rate of NTDs in California showed no decline since fortification. The reduction of the United States overall was 26-30% since folic acid fortification (Imbard et al., 2013).

Folate Receptor Antibody Testing (FRAT®)

Folate deficiency in the pregnant individual can "lead to pregnancy-related complications including neural tube defects (NTDs) in the fetus. Numerous studies have now established the benefits of folate supplementation in reducing the incidence of NTD pregnancy" (Sequeira, 2012). Fratnow's FRAT® measures the "presence of antibodies that interact by either blocking or binding with the activity of the Folate Receptor A. Data shows that folate is critical for the proper function of many tissues, including brain, placenta, and ovaries. FRAT® is not indicated for the diagnosis of any medical condition and thus has not been approved by the FDA. FRAT® can be useful as a research tool in the above disorders, as well as assessing the health of folate transport to the brain, placenta, and ovary" (Fratnow, 2016).

Causes of Folate Deficiency

Folate deficiency can be caused by dietary intake. Nutritional deficits may occur due to diet, alcoholism, depression, and even overcooked foods. Many malabsorptive disorders, such as celiac disease and ulcerative colitis, can also result in a decrease in folate uptake. Further, bariatric procedures may result in decreased absorption, and drugs, including methotrexate and trimethoprim that inhibit dihydrofolate reductase (DHFR), can also cause a folate deficiency. It is also important to note that an increased need of folate for DNA synthesis during pregnancy and lactation, chronic hemolytic anemias, exfoliative skin diseases, and hemodialysis cause folic acid deficiency (Means Jr & Fairfield, 2023b).

Methodology of Folate Testing

Folate Testing, continued

Folate concentrations have been measured from serum, erythrocytes (RBC), and urine. Serum folate levels may not "differentiate between what may be a transitory reduction in folate intake or chronic folate deficiency accompanied by depleted folate stores and functional changes" (IOM, 1998). RBCs have a lifespan of approximately 120 days, and folate is only taken in during initial erythropoiesis (red blood cell production); consequently, RBC folate concentrations are less likely to be affected by transitory dietary fluctuations. However, Wu et al. (1975) show that both RBC folate and serum folate levels correlate to hepatocyte folate levels (IOM, 1998; Wu et al., 1975). Galloway and Rushworth (2003) released a study in conjunction with the National Pathology Alliance review in the United Kingdom comparing data of laboratories of the National Health Service that routinely use serum folate testing only, RBC folate testing only, or both serum and RBC folate testing together. The researchers conclude that there is no need to use both tests to determine folate concentration as an initial screen. "The serum folate assay provided equivalent information to the measurement of red cell folate and evidence from the literatures [sic] suggest that the serum folate assay should be the method of choice" (Galloway & Rushworth, 2003).

Clinical Utility and Validity

A study by Shojania and von Kuster (2010) investigated the use of serum folate testing (Trompeter et al.) and RBC folate testing (RF) in cases of anemia in a country that has mandated folic acid supplementation in grain products. By examining the data for folate testing in anemia at two different teaching hospitals in Canada, they report that in one hospital in 2001 "11 out of 2154 (0.5%) SF were low (<7.0 nmol/L) and 4 out of 560 (0.7%) RF were low (<417 nmol/L). In no subject with low SF or RF could the anemia be attributed to folate deficiency." For the other hospital, the data from 1999-2001 shows that "19 out of 991 (1.9%) had low RF (<225 nmol/L) but in only 2 patients (0.2%) the low RF was in folate deficiency anemia range" (Shojania & von Kuster, 2010). The authors conclude that neither serum folate testing nor RBC folate testing is justified in cases of anemia for folic acid fortified countries due to such low incidence rates of folate deficiency anemia.

Another study by Joelson, Fiebig, and Wu (2007) examined the records of three different hospitals in the U.S. that service a high number of indigent patients. The researchers reported the data from three non-consecutive years (1997, 2000, and 2004) to examine the impact of folate fortification in food products. Using the RBC folate levels only with a RBC folate cutoff value of 160 ng/mL (363.6 nmol/L), "the combined incidence of folate deficiency decreased from 4.8% in 1997 to 0.6% in 2004…Even when the folate concentration was found to be low, the majority of these subjects did not have macrocytosis." This study included a total of 4134 RBC folate tests performed over the course of three years. It is of interest to note that the number of tests performed increased from 813 in 1997 to 1759 in 2004. The authors do note of a potential limitation of the study since the data of the patients cannot be separated into specific groups (pregnant individuals, alcoholics, socioeconomic classes, and so on). The authors conclude "that folate deficiency has become a rare event in the United States, and the utility of routine folate measurements for patients with anemia and/or increased mean corpuscular volume are difficult to justify" (Joelson et al., 2007).

Urinary folate levels do not reflect either the stored folate concentrations or the fluctuations in folate concentration due to transitory dietary changes. Only about 1-2% of the folate excreted in the urine is unmetabolized and "excretion continued in the face of advanced folate depletion" (IOM, 1998). One study of ten postmenopausal individuals on a low folate diet measured folate turnover using urinary testing of folate and folate metabolites. "Folate intake did not significantly influence ApABG (*para*-acetamidobenzoylglutamate) or pABG (*para*-aminobenzoylglutamate) excretion." ApABG and pABG along with pterins are the major folate catabolites. The authors conclude that "the rate of folate catabolite excretion is related mainly to masses of slow-turnover folate pools governed by long-term folate intake" (Gregory et al., 2000).

Epstein-Peterson et al. (2020) collected and analyzed all folate tests performed in 2017 at an academic cancer center. In total, 937 patients were tested 1065 times; approximately 7% of tests indicated a folate deficiency, and folate deficiency was significantly associated with a higher risk of death (P=0.01) (Epstein-Peterson et al., 2020).

Tran et al. (2022) performed a literature review on the diagnostic accuracy, clinical utility, cost-effectiveness, and evidence-based guidelines regarding the use of serum folate testing in people with suspected folate deficiency. An information specialist completed a literature search using the search concepts "folate deficiency AND testing" and only limiting results to the human population for publications between January 1, 2012, and February 15, 2022. The authors were not able to identify any relevant literature regarding diagnostic test accuracy, clinical utility, cost-effectiveness, or evidence-based guidelines (Tran et al., 2022).

IV. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

The CDC urges all individuals who are capable of becoming pregnant and who are also of reproductive age to take "400 micrograms (Handelsman et al.) of folic acid each day, in addition to consuming food with folate from a varied diet, to help prevent some major birth defects of the baby's brain (anencephaly) and spine (spina bifida)" (CDC, 2022). This recommendation includes all individuals of reproductive age planning to become pregnant or not, as about half of U.S. pregnancies are unplanned.

American Society for Clinical Pathology (ASCP)/Choosing Wisely

The ASCP published a recommendation in 2017 in Choosing Wisely, an American Board of Internal Medicine (ABIM) initiative, where they clearly state the following: "Do not order red blood cell folate levels at all. In adults, consider folate supplementation instead of serum folate testing in patients with macrocytic anemia." They indicate that the drastic decrease in folic deficiency in both the U.S. and Canada after mandated folic acid supplementation in foods no longer requires for either serum folate or red blood cell folate concentrations be tested. "While red blood cell folate levels have been used in the past as a surrogate for tissue folate levels or a marker for folate status over the lifetime of red blood cells, the result of this testing does not, in general, add to the clinical diagnosis or therapeutic plan" (ASCP, 2017).

National Pathology Alliance (of the United Kingdom)

Folate Testing, continued

The National Pathology Alliance of the United Kingdom in 2003 published in the *Journal of Clinical Pathology* their recommendation "that serum folate measurements provide equivalent information to red cell folate measurements" (Galloway & Rushforth, 2003).

American Association of Clinical Endocrinologists (AACE)/The American College of Endocrinology (ACE), The Obesity Society (TOS), American Society for Metabolic and Bariatric Surgery (ASMBS), Obesity Medicine Association (OMA), and American Society of Anesthesiologists (ASA)

In 2013, the AACE, ACE, and TOS issued joint guidelines regarding healthy eating for the prevention and treatment of metabolic and endocrine diseases in adults (Gonzalez-Campoy et al., 2013). Based on the data from the National Health and Nutrition Examination Survey (NHANES), they state "that patients with vitamin B_{12} deficiency had higher folate levels, were more likely to be anemic, and had more cognitive impairment that those with normal serum folate levels" [evidence level (EL) 2]. They evaluate the evidence concerning the link between folate and cardiovascular disease as EL4 and the link between NTDs and folate as EL1. With respect to pregnancy nutritional needs, they "should be assessed prior to conception to improve pregnancy outcome…All women of childbearing age should consume at least 400 µg dietary equivalents of folate per day" [EL4] and that during pregnancy the daily amount should be increased to 600 µg [EL3].

The AACE and ACE in 2015 released their *Clinical Practice Guidelines for Developing a Diabetes Mellitus Comprehensive Care Plan* (Handelsman et al., 2015). Concerning patients with diabetic nephropathy, the suggest that they "undergo annual or more frequent assessment of electrolytes". For those with anemia, iron, transferrin saturation (TSAT), ferritin, vitamin B_{12}, and folate levels "should be further investigated" [EL4].

In 2017, the AACE and ACE released their guidelines for management of dyslipidemia and prevention of cardiovascular disease (Jellinger et al., 2017). Since bile acid sequestrant treatments such as cholestyramine can cause folate depletion in children, they recommend that children on such treatments supplement their diet with a multivitamin. They also note that folate, B6, and B12 supplementation can help mediate hyperhomocysteinemia, but that the supplements do not reduce risk of atherosclerotic cardiovascular disease.

In 2019, the AACE/ACE, TOS, ASMBS, OMA, and ASA issued joint guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient (Mechanick et al., 2019). Here, as part of a pre-operative bariatric surgery checklist that has a "Grade A" recommendation, they include "nutrient screening with iron studies, B_{12} and folic acid (RBC folate, homocysteine, methylmalonic acid optional) …consider more extensive testing in patients undergoing malabsorptive procedures based on symptoms and risks." With regards to patients who become pregnant after having a bariatric procedure, they recommend (with Grade D) having nutritional surveillance laboratory screenings done each trimester for folate deficiency along with iron, calcium, B_{12}, and vitamin D, and if after a malabsorptive procedure, fat-soluble vitamins, zinc, and copper. With a Grade C, they state that "nutritional anemias resulting from malabsorptive bariatric surgical procedures can involve deficiencies in vitamin B12, folate, protein, copper, selenium, and zinc and may be evaluated when routine aggressive case finding for iron-deficiency anemia is negative." Additionally, findings of folate deficiency

in patients with obesity prior to bariatric surgery by the ASMBS "justifies aggressive case finding preoperatively with biochemical testing, specifically using sensitive markers, such as red-blood-cell folate and homocysteine (methylmalonic acid is normal with folate deficiency and normal B12 status)" and "particular attention should be given to female patients of childbearing age."

National Institute for Health and Care Excellence (NICE)

The National Institute for Health and Care Excellence (NICE) of the Department of Health in the United Kingdom published their extensive guidelines concerning bladder cancer on February 25, 2015. Within the section concerning the follow-up treatment for muscle-invasive bladder cancer, they recommend a protocol after radical cystectomy that includes "monitoring for metabolic acidosis and B12 and folate deficiency at least annually." (NICE, 2015). This guideline was reaffirmed in 2019.

American Academy of Family Physicians (AAFP)

The AAFP released the recommendations concerning macrocytosis and macrocytic anemia in 2009. Of note, they state that "serum folate levels are not useful because they fluctuate rapidly with dietary intake and are not cost effective. RBC folate levels more accurately correlate with folate stores and should be performed if folate deficiency is suspected." They give the following key recommendation (with evidence rating of "C" or "consensus, disease-oriented evidence, usual practice, expert opinion, or case series") to "obtain red blood cell folate level if other etiologies are not found (serum folate levels may be misleading)." In the evaluation of macrocytic anemia, they included a flowchart outlining the order of steps and tests to be taken, including when the RBC folate level should be checked. For a patient exhibiting a mean corpuscular volume 100 fL and an abnormal peripheral smear showing megaloblastic features and a reticulocyte count under 2%, they should have their RBC folate level measured only if the vitamin B_{12} level is >400 pg. The flowchart is included below (Kaferle & Strzoda, 2009).

Folate Testing, continued

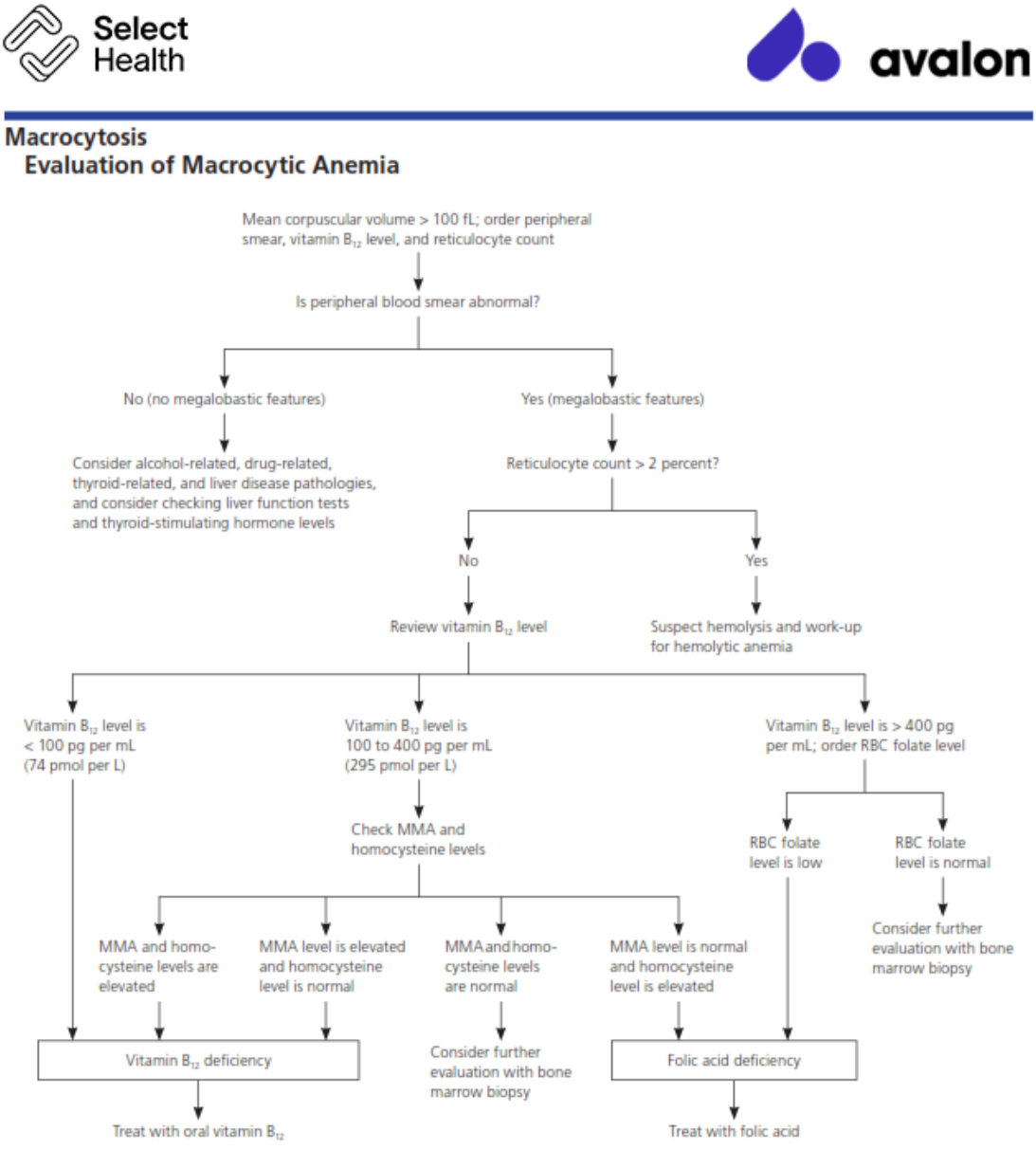

Figure 3. Algorithm for the evaluation of macrocytic anemia. (RBC = red blood cell; MMA = methylmalonic acid.)

American Academy of Neurology (AAN)

In 2001, the AAN updated their practice parameters for the diagnosis of dementia. Within the section concerning the comorbidities that should be screened in an initial assessment for dementia, they recommend folate testing along with complete blood count, serum electrolytes, B_{12}, blood urea nitrogen/creatinine, syphilis serology, thyroid function, and glucose. They did note that as of that time

"no studies were identified that evaluated these recommendations" since the last practice parameters released in 1994.

In 2009, the AAN published guidelines regarding the management and care of women with epilepsy (WWE) during pregnancy. These guidelines state that "Folic acid supplementation is generally recommended to reduce the risk of MCMs [major congenital malformations] during pregnancy, and although the data are insufficient to show that it is effective in WWE, there is no evidence of harm and no reason to suspect that it would not be effective in this group. Therefore, all women of childbearing potential, with or without epilepsy, should be encouraged to take at least 0.4 mg of folic acid daily prior to conception and during pregnancy. There was insufficient published information to address the dosing of folic acid (AAN, 2009)."

Kidney Disease Improving Global Outcomes (KDIGO)

KDIGO released their updated *KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease* in 2012. They gave a "not graded" recommendation for "in patients with CKD [chronic kidney disease] and anemia (regardless of age and CKD stage), include the following tests in initial evaluation of the anemia:

- Complete blood count (CBC), which should include Hb concentration, red cell indices, white blood cell count and differential, and platelet count
- Absolute reticulocyte count
- Serum ferritin level
- Serum transferring saturation (TSAT)
- Serum vitamin B_{12} and folate levels"

They also state that "RBC folate levels can be measured when serum folate levels are equivocal or when there is concern that recent dietary intake may obscure underlying folate deficiency using serum levels alone" (McMurray et al., 2012)

American Society for Parenteral and Enteral Nutrition (ASPEN) & Society of Critical Care Medicine (SSCM)

In 2013, ASPEN and SSCM issued joint clinical guidelines concerning the nutrition support of hospitalized obese adults. With a "Recommendation: Weak" status, they recommended "in acutely ill hospitalized patients with history of these procedures [sleeve gastrectomy, gastric bypass, or biliopancreatic diversion ± duodenal switch], evaluation for evidence of depletion of iron, copper, zinc, selenium, thiamine, folate, and vitamins B_{12} and D is suggested as well as repletion of deficiency states" (Choban et al., 2013).

In 2016, ASPEN and SSCM issued their *Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient*. The committee recommended that "evaluation for and treatment of micronutrient deficiencies such as calcium, thiamin, vitamin B12, fat-soluble vitamins (A, D, E, K), and folate, along with the trace minerals iron, selenium, zinc, and copper, should be considered" (McClave et al., 2016). In 2017, ASPEN and SSCM updated their *Guidelines for the provision and*

assessment of nutrition support therapy in the pediatric critically ill patient. These guidelines do not mention folate testing (Mehta et al., 2017).

Academy of Nutrition and Dietetics (AND)

The AND released their *Oncology evidence-based nutrition practice guideline* in 2013 and reaffirmed the guideline in a 2017 publication. On the "Assessment of Biochemical Data Medical Tests, and Procedures on Adult Oncology Patients" portion, the committee recommended with "Consensus, Imperative" that "the RDN [Registered Dietitian Nutritionist] should evaluate available data and recommend as indicated: biochemical data, medical tests and procedures of adult oncology patients" and included on their list is "Nutritional anemia profile (hemoglobin, hematocrit, folate, B12, iron)". "Assessment of these factors is needed to effectively determine nutrition diagnoses and plan the nutrition interventions" (Thompson et al., 2017)

European Crohn's and Colitis Organisation (ECCO)

ECCO's guidelines concerning irritable bowel disorders (IBD) included an extensive discussion on causes and treatments of anemia in IBD—both iron deficiency anemia and non-iron deficiency anemia. With an [EL 5], they state that "deficiencies of Vitamin B_{12} and folate should be treated to avoid anaemia. Serum levels of vitamin B_{12} and folic acid should be measured at least annually, or if macrocytosis is present. Patients at risk for vitamin B_{12} or folic acid deficiency [e.g., small bowel disease or resection] need closer surveillance. The recommended timelines are based on expert opinions and reflect common clinical practice, but do not apply to patients with extensive small bowel resection, extensive ileal Crohn's disease, or ileal-anal pouch" (Dignass et al., 2015)

American College of Gastroenterology (ACG)

In their guidelines and recommendations concerning the diagnosis and management of celiac disease (CD) in 2013, the ACG recommended the following statement with *Conditional recommendation, low level of evidence*: "People with newly diagnosed CD 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." [Reaffirmed, 2016]

British Committee for Standards in Haematology (BCSH)

In 2014, the BCSH released guidelines on folate deficiencies. They noted that "routine red cell folate testing is not necessary because serum folate alone is sufficient in most cases." However, they also acknowledged that "in the presence of strong clinical suspicion of folate deficiency, despite a normal serum level, a red cell folate assay may be undertaken, having ruled out cobalamin deficiency." The BCSH also noted that "folate status is generally checked in clinical situations similar to those of cobalamin deficiency (Grade 1A)."

In 2016, the BCSH recommended that a "documented vitamin B12 or folate deficiency should be corrected before a final diagnosis of AA is confirmed. Bone marrow aplasia due to vitamin deficiency is exceedingly rare (Killick et al., 2016)."

In the 2021 BCSH *Guidelines for the Investigation and Management of Vitamin B12 and Folate Deficiency* list the following four indications for folate testing: "unexplained anaemia/macrocytic anaemia/megaloblastic anaemia, excess alcohol intake especially with coexisting liver disease, exfoliative skin diseases, post gastric and bariatric surgery." Alternatively, the guidelines list the following two indications when folate supplementation should occur without folate testing: "pregnancy, haemolytic anaemia – autoimmune haemolysis, red cell membrane disorders and haemoglobinopathies." The guidelines also state that folate and B12 should always be tested together, but notes that "once a patient has commenced B12 replacement there is no further need for it to be measured again" (BCSH, 2021).

Renal Association Clinical Practice Guideline

The Renal Association recommends measuring serum folate concentration for evaluation of anemia in CKD (Mikhail et al., 2017).

National Comprehensive Cancer Network (NCCN)

The NCCN recommends measurement of RBC folate as part of the initial evaluation for myelodysplastic syndromes. Serum folate may be considered as an alternative, but is not preferable to RBC folate. "RBC folate is a more representative measure of folate stores and is the preferred test to serum folate" (NCCN, 2023).

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
82746	Folic acid; serum
82747	Folic acid; RBC
0399U	Neurology (cerebral folate deficiency), serum, detection of anti-human folate receptor IgG-binding antibody and blocking autoantibodies by enzyme-linked immunoassay (ELISA), qualitative, and blocking autoantibodies, using a functional blocking assay for IgG or IgM, quantitative, reported as positive or not detected

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
8/23/22	Modified wording in coverage criteria #3 to ensure clarity.
10/24/23	The following changes were implemented: coverage criteria #1 reorganized from having sub-criteria into being a single, main requirement. Now reads: "For individuals diagnosed with megaloblastic or macrocytic or unexplained anemia and for whom the anemia and/or macrocytosis does not resolve after folic acid treatment, measurement of serum folate concentration **MEETS COVERAGE CRITERIA."** Addition of new coverage criteria #4: "4) For all situations, folate receptor autoantibody testing **DOES NOT MEET COVERAGE CRITERIA**."

Folate Testing

Policy #: AHS – G2154	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/23/22, 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

Folate, or vitamin B9, is a generic term for a water-soluble vitamin obtained from the diet that is involved in the transfer of methyl groups (i.e. single carbon-containing groups) in multiple biochemical metabolic pathways, including nucleic acid biosynthesis and methionine/homocysteine metabolism. Folate metabolism is closely linked to vitamin B12, cobalamin. Folate deficiency can be implicated in many disease states and processes; however, it is usually easily remedied with either a change in diet or a dietary supplement of the synthetic form, folic acid (Means Jr & Fairfield, 2023a; NIH, 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 diagnosed with megaloblastic or macrocytic or unexplained anemia and for whom the anemia and/or macrocytosis does not resolve after folic acid treatment, measurement of serum folate concentration **MEETS COVERAGE CRITERIA.**
2. For all indications not described above, measurement of serum folate concentration **DOES NOT MEET COVERAGE CRITERIA**.

Gamma-glutamyl Transferase

Policy #: AHS – G2173	**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

Gamma-glutamyl transferase (GGT), also known as gamma-glutamyl transpeptidase (GGTP) (Singh, Tiwary, Patil, Sharma, & Shukla, 2006; Vroon & Israili, 1990), is an enzyme present in the cell membrane of many different tissue types, including the heart, brain, seminal vesicles, kidneys, bile duct, spleen and gallbladder (Dillon & Miller, 2016). GGT is traditionally considered a predictive marker for liver dysfunction, bile duct ailments and alcohol consumption (Koenig & Seneff, 2015). However, new research has suggested that GGT may be useful as an early predictive marker for several other conditions, including heart failure, arterial stiffness, arterial plaque, gestational diabetes, atherosclerosis, several infectious diseases and numerous types of cancer (Koenig & Seneff, 2015).

NOTE: This policy is intended only for adult individuals aged 18 years and older.

II. Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis C
AHS-G2110	Serum Marker Panels for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease

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. Serum GGT testing* (See Note 1) **MEETS COVERAGE CRITERIA** in individuals with elevated alkaline phosphatase activity.
2. Serum GGT testing* (See Note 1) to assess liver injury, function, and/or disease **MEETS COVERAGE CRITERIA** in individuals who meet at least one of the following:
    a. Chronic alcohol or drug ingestion
    b. Long-term drug therapy known to have a potential for causing liver toxicity
    c. Exposure to hepatotoxins
    d. Viral hepatitis, amoebiasis, tuberculosis, psittacosis, or similar infections that may cause hepatic injury
    e. Primary or secondary malignant neoplasms
    f. Diabetes mellitus
    g. Malnutrition
    h. Disorders of iron and mineral metabolism
    i. Sarcoidosis
    j. Amyloidosis
    k. Lupus
    l. Hypertension
    m. Gastrointestinal disease
    n. Pancreatic disease
    o. As part of liver function assessment subsequent to liver transplantation
3. Serum GGT testing **DOES NOT MEET COVERAGE CRITERIA** as part of a wellness check or for general encounters without abnormal findings.

*Note 1: A maximum of one unit of GGT per week will be reimbursed for adult individuals. In accordance with NCD 190.32, "When used to assess liver dysfunction secondary to existing non-hepatobiliary disease with no change in signs, symptoms, or treatment, it is generally not necessary to repeat a GGT determination after a normal result has been obtained unless new indications are present (CMS, 2019)."

IV. Scientific Background

Gamma-glutamyl transferase (GGT) is a cell surface enzyme found throughout the body. GGT cleaves extracellular glutathione (an antioxidant) and other gamma-glutamyl compounds to increase the availability of amino acids for intracellular glutathione synthesis purposes; GGT also plays an important role in maintaining glutathione homeostasis, as well as in providing defense against oxidative stress (Ndrepepa & Kastrati, 2016). The measurement of circulating GGT is often used as a diagnostic tool for the identification of liver diseases, biliary diseases and alcohol consumption. This is because GGT is very abundant in the liver; considerable GGT concentrations are also found in the intestine, kidney, prostate

and pancreas (Newsome et al., 2018). While GGT measurement may not be useful in the diagnosis of specific types of liver disease, it is one of the best predictors of overall liver mortality (Newsome et al., 2018). Additional research has shown that elevated GGT concentrations in the serum may also be associated with an increased risk of type 2 diabetes, gestational diabetes, hypertension, stroke, coronary heart disease, and cancer (Koenig & Seneff, 2015). Abnormal GGT levels are also identified in anorexia nervosa, Guillain-Barré syndrome, hyperthyroidism, obesity, dystrophica myotonica (Gowda et al., 2009) and cigarette smoking (AACC, 2020). Certain drugs may lead to unusual GGT levels in the blood as well. It has been reported by the AACC (2020) that drugs such as phenytoin, carbamazepine, barbiturates (including phenobarbital), lipid-lowering drugs, antibiotics, antifungal agents, anticoagulants, immunosuppressive medications, antidepressants, hormones, nonsteroidal anti-inflammatory drugs (NSAIDs), oral contraceptives, testosterone, and histamine receptor blockers may cause an increase or decrease in GGT levels. LabCorp (2019) does not recommend ordering a GGT test if the patient is currently taking phenytoin or phenobarbital since these medications may lead to false elevations in GGT.

GGT measurement may also be a useful secondary measure to assist with liver diagnoses. Alkaline phosphatase (ALP) is an enzyme found throughout the body and is typically identified in the liver or bone. Meanwhile, GGT is not found in bone (Singh et al., 2006). Therefore, if elevated ALP levels are detected in a patient, physicians may use a high GGT level to rule out bone disease as the cause of an elevation of ALP; however, if GGT is low or normal, then elevated ALP levels are more likely to be caused by bone disease (AACC, 2020). This means that elevated GGT levels suggest that elevated ALP levels are of a hepatic origin (Kwo, Cohen, & Lim, 2017).

Koenig and Seneff (2015) report that population-wide GGT levels have increased steadily in the United States over the last three decades. This may factor into an increased disease risk over time. It has been hypothesized that GGT levels are increasing due to a greater exposure to environmental and endogenous toxins which result in increased levels of oxidative and nitrosative stress (Koenig & Seneff, 2015). Elevated serum GGT levels are known markers of oxidative stress (Yamada et al., 2006), which occurs when an imbalance is present between antioxidants and free radicals in the body. Simple lifestyle changes, such as avoiding exposure to toxic chemicals and limiting iron intake, may help to lower GGT levels.

Liver function tests are blood tests typically ordered as a panel rather than solitarily. These tests measure the level of several liver enzymes in serum or plasma samples. The liver enzymes frequently measured to detect liver abnormalities include serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), ALP and bilirubin; other liver tests may incorporate the measurement of GGT, albumin and prothrombin time (Kwo et al., 2017). Some report that GGT is only occasionally included in a liver function testing panel (Dillon & Miller, 2016), while others report that GGT is still a commonly measured serum liver enzyme (Friedman, 2020). Nevertheless, Dillon and Miller (2016) conclude that GGT should be measured on liver functioning test panels “some of the time.” This is likely because GGT measurement is not very specific, and its elevation will typically not help the physician to differentiate between diseases.

GGT and Liver-Related Diseases

The liver is an organ in the abdomen which detoxifies metabolites, manufactures proteins and generates biochemicals required for growth and digestion. Many types of liver disease exist, such as hepatitis A, hepatitis B, hepatitis C, cirrhosis, fatty liver disease and liver cancer to name a few. GGT is elevated in the blood in most diseases that cause damage to the liver, including hepatitis and cirrhosis (AACC, 2020). Primary biliary cholangitis (PBC), drug-induced liver injury (DILI), alcoholic liver disease (ALD), and non-alcoholic fatty liver disease (NAFLD) are the main causes of the abnormal GGT in clinic. GGT levels have different characteristics in different liver diseases. For instance, abnormal GGT in PBC and DILI was associated with cholestasis; in ALD, it was associated with both oxidative stress and cholestasis, and in NAFLD, it was associated with oxidative stress (Xing et al., 2022).

Hepatitis C is a viral infection that targets the liver and causes inflammation. An increase in serum GGT levels is seen in approximately 30% of patients with a chronic hepatitis C infection; GGT levels will peak in the second or third week of illness and may remain elevated for up to six weeks (Gowda et al., 2009). Further, the GGT-to-platelet ratio has been identified as a reliable laboratory marker in the prediction of liver fibrosis stage in patients with a chronic hepatitis B infection; this ratio was more reliable than AST-to-platelet ratio index (APRI) and fibrosis-4 score (FIB-4) (J. Lee et al., 2018; R. Q. Wang et al., 2016). The FIB-4 score is a non-invasive scoring system based on several laboratory tests to estimate the amount of scarring in the liver. GGT is also acknowledged as a more specific tool for the identification of non-alcoholic fatty liver disease than ALT (Dillon & Miller, 2016). Finally, GGT has also been identified as a useful prognostic tool for patients with hepatocellular carcinoma, the most common type of primary liver cancer (Z. Wang et al., 2014).

GGT and Bile Duct Diseases

The bile ducts are thin tubes that connect the liver to the small intestine. These ducts help to transport bile from the liver and gallbladder to the small intestine; the bile then assists with the digestion of fats in foods. Singh et al. (2006) report that in 55 patients aged 23 to 45 years, “GGT and ALP levels were normal in patients of chronic cholecystitis with cholelithiasis but significantly high in patients of common bile duct obstruction.”

Biliary atresia (BA) is a childhood disease characterized by absent, narrow or blocked bile duct(s). BA is often identified in infants, and high GGT levels are typically detected. Agin et al. (2016) note that GGT elevation is one of the most reliable tests for diagnosing BA. Other researchers have reported that the value of GGT as a diagnostic tool for BA is largely dependent on age. Specifically, “GGT levels contribute to the diagnosis of BA before 120 days. Age must be considered if using GGT levels as a diagnostic test for BA (Chen, Dong, Shen, Yan, & Zheng, 2016).” GGT was found to have the highest diagnostic value in patients who were between 61-90 days old (sensitivity of 82.8% and specificity of 81.6%), and the lowest diagnostic value in patients who were greater than 121 days old (Chen et al., 2016).

GGT and Kidney/Renal Diseases

The kidneys filter the body's blood by removing waste and maintaining electrolyte balance. Acute kidney or renal injuries are sudden episodes of kidney damage or failure. Lippi et al. (2018) showed that, in dogs with acute kidney injury, significantly higher GGT urine levels were identified.

Chronic kidney disease (CKD) occurs when the kidneys are no longer able to filter blood correctly. Several liver enzyme serum levels, including GGT, have been measured in patients with CKD. However, one analysis reported that relevant GGT data were scant and that "those found reported that there were no differences between the patients with or without chronic kidney disease" (Sette & Almeida Lopes, 2014). Noborisaka, Ishizaki, Yamazaki, Honda, and Yamada (2013) researched elevated serum GGT levels in cigarette smokers and monitored the development of CKD. The authors completed a 6-year retrospective study on 2,603 male workers and concluded that the "elevation of serum GGT in smokers, to a large extent, depends on the associated alcohol consumption. Elevated GGT in smokers plays at least a partial role in the development of CKD, mainly proteinuria, and the underlying mechanisms remain to be elucidated (Noborisaka et al., 2013)."

In another study, the authors claimed that GGT variability may be able to predict the risk of end-stage renal disease (ESRD). GGT variability was assessed using the average successive variability, standard deviation, and CV of serial measurements of GGT during the 5 years before the baseline examination. Subjects were divided into 4 quartiles and those in GGT ASV quartile 4 were older, more obese, and had higher BP and more comorbidities than those in quartile 1. The metabolic variables got worse as the baseline GGT quartile increased. Overall, the implications of GGT levels were statistically significant, especially in women and in ESRD caused by diabetic nephropathy (D. Y. Lee et al., 2020).

GGT and Pancreatic Diseases

The pancreas is in the abdomen and helps to regulate blood sugar and digestion. Several disorders of the pancreas exist, including type 1 diabetes, type 2 diabetes, pancreatic cancer, and pancreatitis. Elevated GGT levels have been used as a prognostic factor to predict survival time in patients with unresectable pancreatic cancer (Engelken et al., 2003).

Pancreatitis occurs when the pancreas becomes inflamed due to its own digestive chemicals. Elevated GGT levels are often identified in patients with acute and chronic pancreatitis (Vroon & Israili, 1990). However, Gori et al. (2019) recently researched the GGT to urinary creatinine ratio in dogs with acute pancreatitis and found no association with any outcome in the study.

GGT and Alcohol Consumption

Increased levels of GGT and alcohol consumption are often correlated. Still, this relationship varies between individuals. GGT concentrations may increase with only small amounts of alcohol consumption in some; on the other hand, only about 75% of chronic drinkers will have elevated GGT levels (AACC, 2020). Nivukoski et al. (2019) report that regular alcohol use is associated with increased GGT and ALT levels. Choe et al. (2019) report that GGT has low sensitivity as a blood biochemical marker of excessive alcohol intake, but the combined use of the CAGE questionnaire (a four-question questionnaire widely

used to screen for alcohol problems) and the measurement of serum GGT is a useful tool for alcohol dependence screening.

GGT and Metabolic Syndrome-Related Risk

Metabolic syndromes are a group of conditions which include high blood sugar, high blood pressure (hypertension), obesity, and abnormal cholesterol levels. GGT has been identified as a biomarker for metabolic syndrome risk (Grundy, 2007). Further, M. Y. Lee et al. (2019) report that GGT levels are significantly higher in subjects with a metabolic syndrome-related disorder than in healthy individuals. Metabolic syndromes collectively increase an individual's risk for the development of many diseases, including heart disease, stroke, type 2 diabetes and neurologic disorders.

- *Cardiovascular Disease*

Cardiovascular disease (CVD), also known as heart disease, encompasses a group of conditions that narrow or block a blood vessel. This may lead to a heart attack, chest pain or stroke. Ndrepepa and Kastrati (2016) previously stated that while more research needs to be conducted, "Ample evidence suggests that elevated GGT activity is associated with increased risk of CVD such as coronary heart disease (CHD), stroke, arterial hypertension, heart failure, cardiac arrhythmias and all-cause and CVD-related mortality. The evidence is weaker for an association between elevated GGT activity and acute ischemic events and myocardial infarction." GGT has been widely identified as a biomarker for cardiovascular risk; in particular, high levels of GGT are associated with a greater risk of atherosclerotic cardiovascular disease (Grundy, 2007), and high GGT variability is associated with an increased risk of myocardial infarction and CVD related mortality (Chung et al., 2019). GGT and the risk of atherosclerosis and coronary heart disease has been reported by Ndrepepa, Colleran, and Kastrati (2018) who report that "it remains unknown whether GGT plays a direct role in the pathophysiology of atherosclerosis and CHD or is merely a correlate of coexisting cardiovascular risk factors." A study by Arasteh et al. (2018) researched how serum GGT can be used as a predictive biomarker for stenosis severity in patients with coronary artery disease; these authors report a significant association between serum GGT activity and patients with coronary artery disease. GGT is considered an inexpensive and readily available biomarker that may provide more information than current tools on the prediction of coronary plaque burdens and plaque structures in young adults (Celik et al., 2014).

- *Cerebrovascular Accident*

A cerebrovascular accident (CVA) or stroke occurs when a blood vessel leading to the brain ruptures or is blocked by a blood clot. There are three main types of CVAs: transient ischemic attack, ischemic stroke, and hemorrhagic stroke. A transient ischemic attack only lasts a few minutes and occurs because of a temporary blood vessel blockage to part of the brain. An ischemic stroke occurs when an artery in the brain is completely blocked, and a hemorrhagic stroke occurs when a ruptured blood vessel causes bleeding in the brain. Several studies have identified a relationship between GGT levels and both hemorrhagic and ischemic CVAs (Korantzopoulos et al., 2009; Xu et al., 2017; Yao et al., 2019).

GGT levels have been associated with functional outcomes after an aneurysms and/or stroke. Xu et al. (2017) state that patients with high GGT levels are more likely to have a poor prognosis after aneurysmal subarachnoid hemorrhage than patients with lower GGT levels, suggesting that serum GGT may be an important prognostic factor for the prediction of aneurysm outcomes. Yang, Kang, and Lee (2020) also report that high GGT levels were significantly associated with cardioembolic stroke through atrial fibrillation (irregular heartbeat). More, GGT variability has been associated with an increased risk of stroke in the general population (Chung et al., 2019), and serum GGT levels have been associated with a greater risk of ischemic or nonembolic stroke in individuals older than 70 years (Korantzopoulos et al., 2009). Serum GGT levels were also found to be significantly elevated in patients who died from an acute ischemic stroke, and high GGT levels were associated with an increased risk of death in male patients with an intracranial arterial calcification (Yao et al., 2019).

- *Type 2 Diabetes*

Type 2 diabetes occurs when the body either does not produce enough insulin, or resists insulin. Diabetes and GGT levels have been researched by Kaneko et al. (2019) who state that the simultaneous elevation of GGT and ALT is significantly associated with the development of type 2 diabetes mellitus; confounding factors include alcohol consumption and obesity. Further, when GGT and ALT were included in type 2 diabetes risk prediction, the accuracy of the prediction was improved (Kaneko et al., 2019). Kunutsor, Abbasi, and Adler (2014) report that greater circulating GGT levels lead to an increased risk of type 2 diabetes in both males and females. Higher GGT levels have also been associated with a greater amount of insulin resistance and therefore a higher risk of developing the disease (Grundy, 2007).

Nano et al. (2017) analyzed 1125 cases of prediabetes and 811 cases of type 2 diabetes. A mendelian randomization (MR) study was performed and the authors found that “MR analyses did not support a causal role of GGT on the risk of prediabetes or diabetes. The association of GGT with diabetes in observational studies is likely to be driven by reverse causation or confounding bias. As such, therapeutics targeted at lowering GGT levels are unlikely to be effective in preventing diabetes (Nano et al., 2017).” This study is important as the results contradict other related studies. Another bidirectional mendelian randomization study analyzed data from 64,094 individuals with type 2 diabetes and 607,012 control subjects; no association between GGT and type 2 diabetes risk was found (De Silva et al., 2019). Further, Shibabaw, Dessie, Molla, Zerihun, and Ayelign (2019) also report that, based on their study, GGT levels were not significantly higher in type 2 diabetes patients compared to healthy controls (P=0.065).

- *Neurodegenerative Diseases*

Abnormal GGT serum levels have been associated with an increased risk of neurodegenerative disease development. Serum GGT levels and Parkinson disease risk in men and women was studied by Yoo et al. (2020) who found that the top quartile of patients with high serum GGT levels was associated with a lower Parkinson disease risk in men and a higher risk in women (n=20,895 Parkinson disease patients). Another study focused on Alzheimer disease showed that alcohol consumption was associated with an

earlier Alzheimer disease age of onset survival and increased GGT blood concentration levels (Andrews, Goate, & Anstey, 2020). Alcohol consumption and GGT levels were not associated with late onset Alzheimer disease risk. Further, Hong et al. (2020) recently reported that GGT variability may lead to an increased risk of all-cause dementia, and Yavuz et al. (2008) found that GGT levels were increased significantly in Alzheimer disease patients in a cross-sectional study of 132 patients with Alzheimer disease and 158 healthy age-matched controls.

Clinical Utility and Validity

Individuals infected with hepatitis C virus are at an increased risk of developing hepatocellular carcinoma even after a sustained virological response is achieved. A total of 642 patients who had achieved a sustained virological response after a hepatitis C infection participated in this study; 33 participants developed hepatocellular carcinoma (Huang et al., 2014). The data showed that "Baseline gamma-glutamyl transferase [GGT] levels strongly correlate with hepatocellular carcinoma development in non-cirrhotic patients with successful hepatitis C virus eradication," suggesting that serum GGT measurement may help to identify specific patients at high risk for developing hepatocellular carcinoma (Huang et al., 2014).

Dong et al. (2018) researched BA (biliary artresia) in a large cohort of Chinese patients. Data from a total of 1728 newborn infants with obstructive jaundice was collected for this study. The authors note that five predictors including "gender, weight, direct bilirubin (DB), alkaline phosphatase (ALP), and gammaglutamyl transpeptidase (GGT) were significantly different between the BA and non-BA groups ($P < .05$) (Dong et al., 2018)." GGT may therefore be an efficient tool for BA diagnoses.

The relationship between liver enzymes and the risk of metabolic syndrome have been researched several times. Liu, Zhou, Lu, Wang, and Qiu (2018) completed a large cross-sectional study with 1444 elderly participants to determine the association between liver enzymes and the risk of metabolic syndrome. The authors noted that "The prevalence of MetS [metabolic syndrome] and its components increased remarkably with increasing quartiles of alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) but not with aspartate aminotransferase (AST) in the elderly," showing that these liver enzymes as positively associated with metabolic syndrome development in elderly populations (Liu et al., 2018). Another study completed by S. Wang et al. (2017) assessed liver function and metabolic syndrome. This study enrolled 32,768 ostensibly healthy participants. Regarding GGT, the authors note that the metabolic syndrome risk "significantly increased… in high quartiles for both genders," suggesting that high GGT levels are a risk factor for the development of metabolic syndromes.

Ndrepepa, Holdenrieder, et al. (2018) compared GGT and ALP to see which was a better prognostic marker for mortality in patients with coronary heart disease. A total of 3768 patients with coronary heart disease participated in this 3-year study. The median value of GGT was 36.2 U/L and the median value of ALP was 69.3 U/L; "Overall, there were 304 deaths: 195 deaths occurred in patients with GGT >median (n = 1882) and 109 deaths occurred in patients with GGT ≤median (n = 1886)… According to ALP activity, 186 deaths occurred in patients with ALP >median (n = 1883) and 118 deaths occurred in patients with ALP ≤median (n = 1885) (Ndrepepa, Holdenrieder, et al., 2018)." The authors conclude that

Gamma-glutamyl Transferase, continued

GGT is a stronger prognostic marker for all-cause mortality in patients with coronary heart disease than ALP.

Conigrave et al. (2002) completed a large, multicenter study with 1863 participants from five countries. This study aimed to measure carbohydrate-deficient transferrin (CDT) and GGT as markers of alcohol consumption. The authors concluded that "CDT was [a] little better than GGT in detecting high- or intermediate-risk alcohol consumption in this large, multicenter, predominantly community-based sample. As the two tests are relatively independent of each other, their combination is likely to provide better performance than either test alone. Test interpretation should take account sex, age, and body mass index (Conigrave et al., 2002)."

Rosoff et al. (2019) studied the association between lipid and liver function enzymes and high-intensity binge drinking (HIBD). This cross-sectional study included 1519 participants. Binge drinking was defined according to the National Institute on Alcohol Abuse and Alcoholism. GGT was one of several enzymes measured (others included high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, total cholesterol (TC), triglycerides (TRG), ALT and AST). The authors state that "HIBD was associated with increased levels of HDL-C, TC, TRG, ALT, AST, and GGT (Rosoff et al., 2019)." Further, the authors also note that the largest increases associated with HIBD was found based on GGT levels, suggesting that "GGT may be most sensitive to HIBD" (Rosoff et al., 2019).

A study completed by Jousilahti, Rastenyte, and Tuomilehto (2000) researched the relationship between serum GGT levels, self-reported alcohol consumption and the risk of stroke. A total of 14,874 participants took part in this study over five years. The authors report that "serum GGT concentration was associated with the risk of total and ischemic stroke in both genders. There was also a significant association among men between GGT and the risk of intracerebral hemorrhage and among women between GGT and the risk of subarachnoid hemorrhage (Jousilahti et al., 2000)." Further, a relationship was not found regarding self-reported alcohol and any type of stroke.

Yang et al. (2020) studied the effects of GGT on stroke occurrence mediated by atrial fibrillation (AF). A total of 880 patients with acute ischemic stroke participated in this study, and AF was identified in 132 of the patients. The authors found that high GGT levels were not associated with large-artery atherosclerosis stroke but were associated with cardioembolic stroke. "The GGT level was significantly associated with cardioembolic stroke via AF. The results obtained in the present study may explain why GGT is associated with stroke (Yang et al., 2020)."

Hong et al. (2020) completed a study to determine if there was a relationship between GGT variability and dementia risk in diabetes mellitus patients. This study included 37,983 diabetic patients who were diagnosed with dementia over a 6.12-year follow-up period. "In the fully adjusted model, the group with the highest quartile of GGT variability had a 19% increased risk of all-cause dementia when compared with the lowest quartile group (Hong et al., 2020)." The authors conclude by stating that in patients with diabetes mellitus, a high amount of GGT variability increased the risk of dementia regardless of other factors such as baseline GGT level.

D. Y. Lee et al. (2020) examined the prognostic value of GGT variability in predicting the risk of stroke, myocardial infarction, and mortality in diabetic patients. 698,937 patients greater than 40 years of age, with a history of diabetes, and without a history of stroke, MI, liver cirrhosis, or chronic hepatitis were included in the study. GGT variability was assessed as the average successive variability (ASV) of serial GGT measurements during the five years before the baseline examination. Subjects were stratified according to quartiles of baseline GGT and GGT ASV. The lower quartile contained subjects with lower GGT levels. According to the results, subjects in GGT ASV quartile 4 were more obese were more likely to have hypertension, dyslipidemia, or chronic kidney disease, and had a higher risk for stroke, MI, and mortality. On the other hand, subjects in quartile 1 were older, and had a higher prevalence of chronic kidney disease but a lower prevalence of hypertension and obesity. The authors conclude that GGT variability is associated with a higher risk of stroke, MI, and mortality; therefore, " it is important to identify the factors that contribute to increased GGT variability to extend the lives of patients with diabetes (D. Y. Lee et al., 2020)."

Mujawar et al. (2020) studied the use of salivary gamma-glutamyl transpeptidase as a biomarker in oral squamous cell carcinoma and precancerous lesions. 75 patients with precancerous lesions or oral squamous cell carcinoma were enrolled in the study and assessed for GGT levels. Healthy participants had a GGT between 4 to 30U/L, those with precancerous lesions had GGT between 39 to 65 U/L, and those with oral squamous cell carcinoma had GGT levels between 53 and 86 U/L. The authors conclude that it can be a reliable biomolecular marker in early detection and prevention of oral cancer that could be routinely employed in dental clinics (Mujawar et al., 2020).

Liao et al. (2022) studied the association of GTT levels with the occurrence of post-stroke cognitive impairment (PSCI). 1,957 participants with a minor ischemic stroke or transient ischemic attack were measured for GTT and they were categorized into 4 quartiles based on baseline GTT levels. Of the 1,957 participants, 671 (34.29%) patients experienced PSCI at 3 months of follow-up. The highest GGT level quartile group exhibited a lower risk of PSCI. The authors conclude that “serum GGT levels are inversely associated with the risk of PSCI, with extremely low levels being viable risk factors for PSCI” (Li, Liao, Pan, Xiang, & Zhang, 2022).

V. Guidelines and Recommendations

American College of Gastroenterology (ACG)

Guidelines from the ACG recommend the following:

- “Before initiation of evaluation of abnormal liver chemistries, one should repeat the lab panel and/or perform a clarifying test (e.g., GGT if serum alkaline phosphate is elevated) to confirm that the liver chemistry is actually abnormal. (Strong recommendation, very low level of evidence).
- An elevation of alkaline phosphatase should be confirmed with an elevation in GGT. Given its lack of specificity for liver disease, GGT should not be used as a screening test for underlying liver disease in the absence of other abnormal liver chemistries. (Strong recommendation, very low level of evidence).

Gamma-glutamyl Transferase, continued

- An elevated alkaline phosphatase level of hepatic origin may be confirmed by elevation of gamma-glutamyl transferase (GGT) or fractionation of alkaline phosphatase
- Measurement of GGT may represent a complementary test to identify patterns of alcoholism or alcohol abuse, although GGT by itself is not helpful in establishing a diagnosis of alcoholic liver disease
- If the alkaline phosphatase is elevated in the presence of other elevated liver chemistries, confirmation of hepatic origin is not required. With isolated alkaline phosphatase elevation, confirmation with GGT, or fractionation of alkaline phosphatase isoenzymes can be used to help differentiate liver alkaline phosphatase from non-liver sources. However, GGT elevation is not specific for cholestatic liver disease, and can be elevated in >50% of alcoholic patients without obvious evidence of liver disease. GGT can also be elevated in patients with pancreatic disease, myocardial infarction, renal failure, emphysema, diabetes, and in patients taking certain medications such as phenytoin and barbiturates. Given its lack of specificity for liver disease, GGT should not be used as a screening test for underlying liver disease in the absence of abnormal liver chemistries
- Those who present with an elevation in alkaline phosphatase with normal AST, ALT, and bilirubin levels should have their alkaline phosphatase elevation confirmed with a GGT level and if elevated an ultrasound of the liver should be ordered (Kwo et al., 2017)."

British Society of Gastroenterology (BSG)

The BSG's guidelines on the management of abnormal liver blood tests state that "Initial investigation for potential liver disease should include bilirubin, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT), together with a full blood count if not already performed within the previous 12 months. (level 2b, grade B) (Newsome et al., 2018)."

European Association for Study of Liver (EASL)

The EASL published clinical practice guidelines for drug-induced liver injuries (DILI). These guidelines state that "ALT, ALP and TBL [total bilirubin] are the standard analytes to define liver damage and liver dysfunction in DILI. AST [aspartate aminotransferase] values can be used to reliably substitute ALT in calculating the pattern of injury when the latter is unavailable at DILI recognition, whereas GGT is less reliable as an ALP substitute. Grade C (Andrade et al., 2019)."

The EASL also published clinical practice guidelines for the management of alcohol-related liver disease (ALD). These guidelines state that "As the measurement of GGT, ALT, AST and MCV [mean corpuscular volume] is easy and inexpensive, they remain the most frequently used markers for early detection of ALD. However, all these laboratory values are only indirect markers for ALD, with low sensitivity and specificity... No single marker or combination of markers can differentiate between different causes of liver disease (Thursz et al., 2018)." The authors also note that "Screening investigations should not only include liver function tests (LFTs), i.e. gamma glutamyl transpeptidase (GGT), serum ALT and serum AST, but also performance of a test to detect liver fibrosis (e.g. TE [transient elastography]) (Thursz et al., 2018)."

European Association for Study of Liver (EASL) and Latin American Association for the Study of the Liver (ALEH)

Guidelines from the EASL and ALEH state that "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 (Castera et al., 2015)." The guidelines provide a list of several serum biomarkers including GGT.

European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)

An expert committee for the ESPGHAN published guidelines for the diagnosis and treatment of nonalcoholic fatty liver disease in children (0-18 years). These guidelines include a section on additional testing to consider for chronic liver diseases. Regarding screening labs, the ESPGHAN recommends considering testing for "CBC [complete blood count] with differential, AST, bilirubin (total, conjugated), alkaline phosphatase, GGT, INR [international normalized ratio], albumin, total protein, [and] hemoglobin A1c" (Vos et al., 2017).

The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)

The NASPGHAN and ESPGHAN published joint guidelines for the evaluation of cholestatic jaundice in infants. While the background of these guidelines does mention GGTP, the official society recommendations do not mention the measurement of GGTP for the evaluation of cholestatic jaundice in infants. The NASPGHAN and ESPGHAN do recommend that "Any formula-fed infant noted to be jaundiced after 2 weeks of age should be evaluated for cholestasis with measurement of total and conjugated (direct) serum bilirubin (1A). Depending upon local practice, breast-fed babies that appear otherwise well may be followed clinically until 3 weeks of age, at which time if they appear icteric should then undergo serum evaluation of total and conjugated (direct) serum bilirubin (Fawaz et al., 2017)."

The background of the article states that "During the evaluation of the infant with cholestasis, laboratory investigations will help define the etiology, the severity of the liver disease and detect treatable conditions. A critical and important initial blood test is the measurement of serum conjugated (direct) bilirubin (DB), which, if elevated, is a reliable laboratory indicator of cholestasis at this age. Accompanying evaluation of DB levels are standard biochemical and synthetic liver tests to assess the severity of the liver disease to include TB, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGTP), prothrombin time (PT) with the international normalized ratio (INR), glucose, and albumin (Fawaz et al., 2017)."

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

The NASPGHAN published guidelines in 2004 for the evaluation of cholestatic jaundice in infants. These guidelines state that "GGTP [gamma-glutamyl transpeptidase] and lipoprotein X are not routinely recommended in the evaluation of cholestasis in young infants (Moyer et al., 2004)." More, this article states that "Gamma glutamyl transpeptidase (GGT) has been used in the past to distinguish biliary atresia from neonatal hepatitis, but wide variability in levels makes interpretation of test results difficult.

Especially in the older infant with cholestasis, a very low GGT level may be useful to exclude obstruction and, in conjunction with an elevated alkaline phosphatase level, suggests genetic and metabolic causes of intracellular cholestasis. The degree of elevation of GGT is not useful in discriminating the etiology of the cholestasis (Moyer et al., 2004)."

Canadian Association of Gastroenterology (CAG)

The CAG practice guidelines for the evaluation of abnormal liver enzyme tests state that GGT may be used as a second-line biochemical test. Specifically, the guidelines state that "All patients with at least one abnormal liver screening test (abnormal ALT, AST or ALP) should have the following liver biochemical tests performed: gamma-glutamyl transferase (GGT), albumin, bilirubin (including direct if the total bilirubin is elevated) and either prothrombin time (PT) or international normalized ratio (INR). These tests can be performed as initial screening tests if it is inconvenient for the patient to return to the physician's office within a reasonable period of time (weeks or months depending on the severity of the enzyme abnormalities) (Minuk, 1998)."

American Society of Addiction Medicine (ASAM)

ASAM released clinical practice guidelines on the use of laboratory tests which measure impairment of hepatic functioning. ASAM recommends measurement of GGT and ALT to identify recent heavy alcohol use and risk for alcohol withdrawal. When using a urine test, GGT is recommended as the marker of heavy alcohol consumption (ASAM, 2020).

British Society of Paediatric Gastroenterology Hepatology and Nutrition (BSPGHAN) (BSPGHAN)

BSPGHAN released guidelines on diagnosis of non-alcoholic fatty liver disease (NAFLD). BSPGHAN recommends GGT as a first line liver function assessments including other such as ALT, AST, ALP, split bilirubin, FBC, coagulation screen, albumin, fasting lipid profile, immunoglobulin and complement levels, autoimmune profile including ANCA, anti-transglutaminase antibodies, thyroid function tests, A1AT levels, copper and caeruloplasmin, and Hepatitis A, B, C, and E serology. BSPGHAN also recommends assessing for GGT at each clinic follow up (BSPGHAN, 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
82977	Glutamyltransferase, gamma (GGT)

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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General Inflammation Testing

Policy #: AHS – G2155	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/24/22, 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

Inflammatory response can occur due to tissue injury and/or various disorders, including arthritis, lupus, and infection. Acute phase reactants, such as serum C-reactive protein (CRP), are released in the acute phase response during inflammation and can be used to monitor inflammation. Inflammation may also be measured using the simple laboratory technique of erythrocyte sedimentation rate (ESR) (Kushner, 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) Measurement of C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR) **MEETS COVERAGE CRITERIA** for inflammatory conditions as specified in Note 1.

2) For individuals without a diagnosed inflammatory condition, measurement of ESR **DOES NOT MEET COVERAGE CRITERIA**.

3) Measurement of CRP and/or ESR during general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA.**

General Inflammation Testing, continued

NOTES:

NOTE 1: Coverage of ESR, CRP, or both CRP and ESR is designated based on the diagnosed or suspected inflammatory condition.

Condition	Test Preference	Frequency of Testing
Acute and Chronic Urticaria	CRP and/or ESR	Not specified (NS)
Acute Hematogenous Osteomyelitis (AHO)	CRP	To confirm diagnosis; 2 to 3 days during the early therapeutic course; weekly until normalization (or a clear trend toward normalization is evident)
Acute Phase Inflammation	CRP	NS
Ankylosing Spondylitis	CRP or ESR	Regular interval use in patients with active symptoms
Arthritis	CRP and ESR	1-3 months initially; 6-12 months later
Castleman's Disease	CRP or ESR	NS
General Inflammation	CRP	NS
Giant Cell Arteritis	CRP and ESR	To confirm diagnosis; during follow-up visits
Hodgkin Lymphoma	ESR	Every 3 to 6 months for 1 to 2 years and then every 6 to 12 months for the next 3 years and then annually
Irritable Bowel Syndrome	CRP and ESR	During initial assessment to exclude other diagnoses
Large Vessel Vasculitis	CRP or ESR	NS
Nonradiographic axial sponyloarthritis	CRP or ESR	Regular interval use in patients with active symptoms
Polymyalgia Rheumatica	CRP and ESR	At initial diagnosis; every 3 months during long-term steroid therapy
Periprosthetic Joint Infections (PJI)	CRP and ESR	NS
Rheumatoid Arthritis	CRP or ESR	Prior to treatment; every 1-3 months during active disease; annually when disease is inactive
Systemic Lupus Erythematosus	CRP and/or ESR	At initial assessment; every 1-3 months during active disease; every 6-12 months during stable disease; during pregnancy
T-cell lymphomas	ESR	NS

III. Scientific Background

Conditions Associated with Acute Inflammatory Responses

Diseases most associated with an acute inflammatory response measured by C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR) include arthritis, especially rheumatoid arthritis (RA), polymyalgia rheumatica (PMR), giant cell arteritis (GCA), systemic lupus erythematosus (SLE), cardiovascular disease (CVD) (Kushner, 2023), and Hodgkin lymphoma (HL) (NCCN, 2022). RA is a systemic polyarthritis that can lead to joint loss as well as tendon and ligament deformation to the point of affecting day-to-day living. The diagnosis of RA can be made in a patient "with inflammatory arthritis involving three or more joints, positive RF [rheumatoid factor] and/or anti-citrullinated peptide/protein antibody, disease duration of more than six weeks, and elevated CRP or ESR, but without evidence of diseases with similar clinical features" (Baker, 2023). PMR "is an inflammatory rheumatic condition characterized clinically by aching and morning stiffness about the shoulders, hip girdle, and neck (Salvarani & Muratore, 2023)." PMR is frequently associated with GCA (also known as Horton disease), which is vasculitis of medium-to-large blood vessels and can include the aorta and cranial arteries. Cranial arteritis can lead to permanent vision loss. An estimated 40-50% of patients with GCA also suffer from PMR whereas 15% of all PMR patients are also diagnosed with GCA. Due to inflammation of the aorta and aortic branches, aortic aneurysm and aortic dissection can occur in patients with GCA (C. Salvarani & F. Muratore, 2023). In both PMR and GCA, ESR and CRP levels are typically elevated. SLE "is a complex autoimmune disease with chronic relapsing-remitting course and variable manifestations leading a spectrum from mild mucocutaneous to devastating, life-threatening illness… Epigenetic modifications mediate the effect of the environment on immunologic responses, eventually leading to an inflammatory, autoimmune, multi-systemic disease characterized by autoantibody production and tissue injury (Gergianaki & Bertsias, 2018)." Since patients with SLE can be prone to infection, ESR and CRP may be used in monitoring inflammation (Kushner, 2023). CVD is a very common inflammatory disorder in the United States. Although serum CRP is a non-specific inflammatory marker and is not a causative agent of CVD, serum CRP can be used as a biomarker for CVD (Black et al., 2004; Kushner, 2023). Hodgkin lymphoma accounts for 10% of lymphomas and is characterized as a B-cell lymphoma "containing a minority of neoplastic cells (Reed-Sternberg cells and their variants) in an inflammatory background" (Aster & Pozdnyakova, 2023). ESR is elevated in HL, and an ESR ≥50 is considered as an "early-stage unfavorable factor" (NCCN, 2022).

Erythrocyte Sedimentation Rate (ESR)

Erythrocyte sedimentation rate (ESR) is a common laboratory method used to monitor general inflammation. ESR is used to analyze many different conditions, including RA, SLE, arteritis, PMR (Kushner, 2023; Wu et al., 2010). The simple Westergren method of ESR consists of measuring the distance a blood sample travels in a tube within one hour. The International Council for Standardization in Hematology (ICSH) established a calibration reference to this method using citrate-diluted samples. Automated ESR methods have been established; however, some of these analyzers use different dilution solutions, such as EDTA, rather than citrate. EDTA is commonly used as an anticoagulant in hematology

measurements whereas the use of citrate is less prevalent. Horsti et al. (2010) compared blood samples from 200 patients using the traditional Westergren method versus an EDTA-based method. Their data has an R^2 value of only 0.72 and 55 subjects had a difference of over 30%, clearly indicating that ESR is significantly affected by sample preparation methods (Horsti et al., 2010). ESR can also be affected by red blood cell morphology, ambient conditions (such as high room temperature or tilting of the ESR tube), anemia, renal disease, obesity, heart failure, and hypofibrinogenemia (Kushner, 2023; Taylor & Deleuran, 2020).

More, ESR may be affected by noninflammatory factors, thus reducing its specificity for inflammatory processes. Noninflammatory biological factors and environmental conditions can increase a sample's observed ESR. If the serum sample contains elevated concentrations of ions or charged proteins, an elevated ESR may occur; for example, an increase in positively charged plasma proteins could result in agglutination of erythrocytes within a sample for rapid sedimentation (Hale et al., 2019).

The ICSH established a Working Group to investigate the ESR methodology used in laboratories; the findings of this working group were published in 2017. Data from over 6000 laboratories on four different continents was examined. Of the laboratories included in the study, only 28% used the "gold standard" Westergren method exclusively (i.e., the method with the established validation by the ICSH) "while 72% of sites used modified or alternate methods." The data obtained from the new methodologies could deviate from the Westergren method by up to 142% and could differ "from each other of up to 42%." The ICSH released recommendations based up the results of these studies. One such recommendation for labs using the non-Westergren method of ESR is to "consider adding an interpretative comment to every result stating that 'This result was obtained with an ESR instrument that is not based on the standard Westergren method. The sensitivity and specificity of this method for various disease states may be different from the standard Westergren method'" (Kratz et al., 2017).

Besides the Westergren method, other methods have been developed to measure ESR including the Zeta sedimentation ratio, Wintrobe's method, and micro-ESR. In a validation study, Shaikh discussed the use of the Ves-Matic Cube 30 analyzer to address the drawbacks of the Westergren method such as contamination risk, the significant blood volume required, and increased duration of analysis. A strong positive correlation was observed between Westergren and Ves-Matic methods with Spearman's coefficient of 0.97. The study concluded that Ves-Matic Cube 30 analyzer can be used in high workload clinical settings for ESR measurement as the generated results were in concordance with those from the Westergren method.

C-reactive Protein (CRP)

C-reactive protein (CRP) was first discovered in the early twentieth century when it was isolated in a co-precipitation reaction with the pneumococcal C polysaccharide. The polysaccharide component bound by CRP later was identified to be phosphocholine. Since then, studies have shown that CRP can bind ligands other than bacterial cell wall components. During an acute inflammatory response, hepatocytes can upregulate CRP synthesis more than 1000-fold. The increase in serum CRP "after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response" (Black et al., 2004). Determining CRP concentration and fluctuations in plasma CRP can be useful in

monitoring inflammatory response; however, what dictates "normal" CRP levels is of debate since CRP concentrations can vary considerably between individuals, people groups, and laboratory testing methodology. The units used to denote CRP concentrations also vary between laboratories (Kushner, 2023).

Clinical Validity and Utility of CRP and ESR in Measuring Inflammatory Processes

Both CRP and ESR have been used to monitor RA. Elevated CRP and ESR does correlate to observed radiologic damage in RA. Unlike ESR, CRP can be evaluated in stored serum. This could be advantageous due to the time constraints of ESR testing (Taylor & Deleuran, 2023). A 2009 study by Crowson et al. (2009) show that the use of both ESR and CRP testing in the case of RA is not warranted. Data from three randomized trials of 1247 RA patients was examined. "Where available, the CRP alone may be preferred for disease activity assessment as a simple, validated, reproducible, non age-dependent test" (Crowson et al., 2009). Since both ESR and CRP have been incorporated into composite scoring for RA, the elimination of one or the other will not hinder the quantitative evaluation of the patient using a composite scoring system such as DAS (Disease Activity Score) or SDAI (Simplified Disease Activity Index). A 2015 Danish study clearly shows that the data obtained in DAS using either ESR or CRP "are interchangeable when assessing RA patients and the two versions of DAS28 are comparable" (Nielung et al., 2015). This study compared the baseline data and one-year follow-up of 109 different patients with RA using the DAS28-ESR and DAS28-CRP. Using the EULAR (European League Against Rheumatism) response criteria, only 14 patients show a divergence between using the ESR and CRP methods. Of those 14, "12 showed a better response (in terms of responder category) using DAS28-CRP, while two patients showed a better response using DAS28-ESR." However, a 2006 study by Fransen and van Riel (2006) show that it is still possible for a patient to have a high number of swollen joints and yet receive a low DAS28-ESR score within the remission range due to a low ESR value since ESR has a significant weight on the DAS28-ESR algorithm (Fransen & van Riel, 2006). This study did not include CRP measurements to compare its validity to that of the DAS28-ESR. Another study released in 2010 (Hensor et al., Conaghan, 2010) shows that the DAS28-CRP could also underestimate RA remission rates since those values are usually lower than the corresponding DAS28-ESR values, but the discrepancy is not significant if age and gender are added as factors into the DAS28-CRP methodology. To confound issues, "newer biologic agents that target specific inflammatory cytokines are differentially reflected in the ESR and CRP and may therefore disproportionately deflate the composite score (Anderson et al., 2012)."

Erythrocyte sedimentation rate cannot be used to predict RA as a screening method. Suarez-Almazor and colleagues investigated the predictive value of ESR for connective tissue diseases (CTD) and RA. Their review of 711 records by more than 300 different primary care physicians in Alberta show that ESR positively predicted 35% for CTD and only 17% for cases of RA. For SLE, the positive predictive value for ESR was even lower at only 3%. CRP testing was not included in this study. The authors note that "most tests were negative, and were often requested in patients without CTD, resulting in low positive predictive values and questionable clinical utility" (Suarez-Almazor et al., 1998). A study by Keenan, Swearingen, and Yazici (2008) compared the utilization of ESR and CRP in RA, SLE, and osteoarthritis. The data showed that for the 188 patients with RA, the number of patients with both ESR and CRP elevated were statistically the same as those with normal test levels or those with only one test elevated.

General Inflammation Testing, continued

Conclusions stated "that another look at the role of ESR and CRP as markers of inflammation in RA patients seen in routine care may be in order (Keenan et al., 2008)."

Bitik et al. (2015) researched the use of elevated ESR and CRP levels in distinguishing the definitive diagnosis of a rheumatic disorder from patients with nonspecific inflammation. In their study of 112 patients, 47 had a previously diagnosed rheumatic disorder and 65 had no history of a rheumatism. Of the 65 patients with no history of a rheumatic disorder, 52.3% were diagnosed with a new rheumatic disorder with PMR/GCA comprising 38.2%, while 47.7% had a non-rheumatic diagnosis. Within this latter group, only the "CRP levels were significantly higher in infections when compared with new onset RD (rheumatic disease) or malignancies ($p < 0.05$) (Bitik et al., 2015)." The ESR levels between the three groups were statistically insignificant. This indicates that CRP is more sensitive to acute infections than ESR. The authors state that "although ESR and CRP levels have a very low specificity in differentiating between these conditions, in cases of unusually high levels of CRP (especially above 200), more consideration should be given to infections or malignancies."

A 2014 study of 60 different PMR patients compared the efficacy of ESR and CRP in assessing disease activity versus patient-reported outcomes and plasma fibrinogen. In this study, the VASDA (Visual analog scale disease activity) and VASQOL (VAS quality of life), two patient-reported outcome methods, were the most responsive to changes in disease activity. Of the serum biomarkers, fibrinogen, ESR, and CRP, fibrinogen was the most accurate with a correlation coefficient of 1.63 whereas 1.2 and 1.05 were the correlation coefficients of ESR and CRP, respectively. These data suggest that plasma fibrinogen would be a more sensitive measure of PMR disease activity as compared to either ESR or CRP (McCarthy et al., 2014).

A two-year retrospective study released in 2010 (Ernst, Weiss, Tracy, & Weiss, 2010) researched the validity of using either ESR and/or CRP in assessing septic joints. This study consisted of 163 patients and included both genders as well as patients with alcohol or drug histories. The mean ESR value for the 119 control non-septic joints was 46 while the septic joint mean ESR value was 57, which was however, the mean CRP value was 13 in the septic joints and 8.5 in the non-septic joints. The conclusion of the authors is that "CRP is helpful in determining the presence of a septic joint; ESR is not (Ernst et al., 2010)."

Erythrocyte sedimentation rate is used in determining the algorithm to follow in the treatment of Hodgkin lymphoma (CHL). For example, in stage 1A CHL, a patient with an ESR <50 would follow either the NCCN HODG-3 or HODG-4 algorithm with an initial 2-3 cycles of ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine) most likely whereas a stage 1A patient with an ESR ≥50 would follow the NCCN HODG-6 algorithm with a possible involved-site radiation therapy (ISRT) initially along with the chemotherapy since an ESR ≥50 is considered an "unfavorable factor" (NCCN, 2022).

CRP elevation is associated with a number of inflammatory disorders (including RA), tissue damage (such as after a myocardial infarction), as well as bacterial infections; however, CRP levels in SLE do not mirror disease progression (Kushner, 2023). Even during cases of severe disease phenotypes, CRP levels can be normal to modestly increased. One possible reason is CRP suppression by type I interferons, which are increased in SLE. Another possibility is that low concentrations of wildtype CRP play a role in lupus.

General Inflammation Testing, continued

"Three lines of investigation have raised the possibility that low plasma levels of CRP may be related to the pathogenesis of SLE: 1) an association between SLE and several CRP genetic polymorphisms, at least one of which is associated with low CRP levels, 2) the possibility that low CRP levels may contribute to defective clearance of autoantigens during apoptosis, and 3) the therapeutic efficacy of CRP in mouse models of SLE (Gaitonde et al., 2008)." Also, CRP and anti-CRP may form large complexes in patients with SLE, which could also decrease the serum concentrations of free CRP (Gordon et al., 2018). A study by O'Neill and colleagues in 2010 show that anti-CRP levels are directly proportional in an increase to disease activity (32.6, 24.8, and 16.8 AU, respectively, for high activity, low activity, and control groups) and that anti-CRP levels were above the upper limit of normal in 26.3% of the high activity cases versus only 12.8% for the low activity cases (O'Neill et al., 2010). Patients with SLE usually have elevated ESR, but this elevation may be due to persistent polyclonal hypergammaglobulinemia (increased production of several different immunoglobulins) (Gordon et al., 2018).

Periprosthetic joint infections (PJI) may also benefit from testing of CRP and ESR. Joint arthroplasties (replacements) are typically performed in response to joint damage or destruction and commonly involve areas such as the hip, knee, or shoulder. Up to 2% of total knee replacements may become infected. Common signs of infection are present in PJI such as joint pain or warmth at the incision site, and microbiological cultures may be performed to confirm the diagnosis. CRP and ESR have been suggested as supportive biomarkers in cases where a definitive diagnosis cannot be made. CRP and ESR are considered minor clinical diagnostic criteria in some definitions of PJI, but due to the ubiquity of these markers, their levels are usually interpreted cautiously (Berbari et al., 2021).

Berbari et al. (2010) performed a meta-analysis of inflammatory markers in prosthetic joint infection. A total of 30 studies including 3909 revision total hip or knee replacements were assessed, and of the 3909 operations, 1270 infections occurred. CRP was included in 23 of 30 studies, and its diagnostic odds ratio was found to be 13.1. ESR was included in 25 of 30 studies, and its diagnostic odds ratio was calculated to be 7.2. Interleukin-6 was found to be the best marker of all markers addressed, albeit with only three studies (Berbari et al., 2010).

Perez-Prieto et al. (2017) examined the performance of CRP and ESR for PJI diagnosis. A total of 73 patients were included in the study. Preoperative CRP levels were found to be normal in 23 patients, and of those 23 patients, 17 patients also had normal ESR levels. Further, 16 patients with normal CRP levels were found to have "low-virulence" organisms (such as *Propionibacterium acnes* and coagulase-negative staphylococci) present. Overall, the authors found that 23% of the patients included in this study would not have been diagnosed with PJI according to the American Association of Orthopaedic Surgeons (AAOS) guidelines or the Musculoskeletal Infection Society definition (Perez-Prieto et al., 2017).

Kheir et al. (2018) evaluated the accuracy of inflammatory markers in diagnosis periprosthetic joint infections (PJI). A total of "549 periprosthetic joint infection cases and 653 aseptic total joint arthroplasty revisions" were reviewed. The sensitivity of ESR to diagnose PJI was 0.85 and 0.88 for CRP. ESR was also elevated in antibiotic-resistant strains of bacteria compared to culture-negative cases. For CRP, gram-negative species had higher levels of CRP than culture-negative cases. Overall, the authors

General Inflammation Testing, continued

concluded that both ESR and CRP had higher false-negative levels than previously reported (Kheir et al., 2018).

Hamann et al. (2019) compared the DAS28-ESR and DAS28-CRP to determine the impact on disease activity stratification in RA. A total of 31,074 paired data sets were included in this study and were obtained from the British Society for Rheumatology Biologics Register for RA. Results showed that "DAS28-CRP scores were ~0.3 lower than DAS28-ESR overall, with greatest differences for women (-0.35) and patients over 50 years old (-0.34). Mean male DAS28-CRP scores were 0.15 less than corresponding DAS28-ESR scores (Hamann et al., 2019)." When DAS28-CRP data is adjusted by gender, significant agreement (P<0.001) is seen with DAS28-CRP and DAS28-ESR scores.

Bingham et al. (2019) measured the specificity and sensitivity of ESR and CRP when screening for a PJI infection using the standard MSIS cutoff of 30 mm/h and 10 mg/L, respectively. The researchers also hoped to determine the optimal CRP and ESR cutoff to achieve a ≥95% sensitivity. Data from a total of 81 PJI patients and 83 noninfected arthroplasty patients was analyzed for this study. Results showed that "The ESR cutoff that resulted in a sensitivity ≥ to 95% (95% CI: 85.2-97.6%) was 10 mm/h, and the CRP cutoff that resulted in a sensitivity ≥ to 95% (95% CI: 87.1-98.4%) was 5 mg/L. The sensitivity and specificity with a combined ESR and CRP of 10 mm/h and 5 mg/L was 100% (Bingham et al., 2019)." The authors note that the accepted cutoff of 30 mm/h and 10 mg/L leads to a high number of false positives and low sensitivity; these thresholds therefore need to be reevaluated.

In a prospective cohort study, Watson et al. (2019) compared the diagnostic value of CRP and ESR and evaluated whether measuring two inflammatory markers increases accuracy. For each test, sensitivity, specificity, PPV, NPV, and AUC were calculated. 136,961 patients with inflammatory testing were measured of which 61.2% had a single marker measured and 38.8% had multiple markers measured. CRP and ESR were broadly similar in terms of sensitivity, specificity, PPV, and NPV. However, CRP had the highest overall AUC of 0.682 while the AUC for ESR was 0.589. Adding a second test did little improvement in AUC. When CRP and ESR were both tested for, the AUC increased from 0.682 to 0.688. Overall, the authors conclude that "Testing multiple inflammatory markers simultaneously does not increase ability to rule out disease and should generally be avoided. CRP has marginally superior diagnostic accuracy for infections, and is equivalent for autoimmune conditions and cancers, so should generally be the first-line test (Watson et al., 2019)."

In a cross-sectional study, Sherkatolabbasieh et al. (2020) investigated platelet count, ESR, and CRP levels in pediatric patients with inflammatory disease. A total of 150 children (75 male and 75 female) with diagnosis of infectious and inflammatory diseases were included in the study. Platelet count, ESR, and CRP levels were measured at the time of hospitalization and at discharge. At time of hospitalization, all 150 children had abnormal ESR levels, 73.3% had abnormal CRP levels, and 8% had abnormal platelet levels. At time of discharge, only one patient recovered to normal ESR levels, 88% had normal CRP, and 93.3% had normal platelet count. The Fisher exact test showed a significant relationship between platelet count and CRP levels at the time of discharge ($p < 0.0002$) and admission ($p < 0.007$), especially in the female patients. CRP levels were significantly higher in the female patients and changes in platelet count were more prevalent. No relation between platelet count and ESR was observed at admission and

discharge. This study found that there are differences in inflammatory markers between the two sexes. The authors conclude that this study showed significant correlation between CRP and platelet levels in girls and CRP level measurement is useful in treatment follow up (Sherkatolabbasieh et al., 2020).

Alende-Castro et al. (2021) studied the use of CRP vs ESR in 1472 patients with no inflammatory conditions. All participants were measured for ESR, CRP and IL-6 concentrations. 74.9% of participants showed normal CRP and ESR values, 4.6% showed high ESR and CRP values, and 13.8% showed high CRP but normal ESR values. Participants with high ESR/CRP values also were of older age, and reported high alcohol consumption, low physical activity, high BMI, and the presence of metabolic syndrome. In those patients who had high CRP but normal ESR, BMI seemed to be the main determinant of CRP concentrations. The authors concluded that "In this general adult population with no overt inflammatory disease, the discordant pattern of high ESR and normal CRP was associated with greater age, whereas the pattern of high CRP and normal ESR was associated with higher BMI" (Alende-Castro et al., 2021).

In a retrospective study, Christopher studied the use of ESR/CRP ratio to differentiate acute vs chronic periprosthetic joint infections. A total of 147 patients (81 acute and 66 chronic) were measured for ESR and CRP concentrations. The mean ESR / CRP ratio in acute patients was 0.48 compared to 2.87 in chronic patients. The ideal cutoff value was 0.96 for ESR / CRP to predict a chronic (>0.96) vs. acute (<0.96) PJI. The sensitivity at this value was 0.74 and the specificity was 0.90. The authors conclude that "The ESR / CRP ratio may help determine the duration of PJI in uncertain cases. This metric may give arthroplasty surgeons more confidence in defining the duration of the PJI and therefore aid in treatment selection" (Christopher et al., 2021).

Dhudasia et al. (2022) conducted a retrospective cohort study to determine the clinical utility of CRP in diagnosing early-onset sepsis and assessing patient outcomes. The patient sample included over 10,000 infants admitted to the neonatal intensive care units from 2009-2014, when CRP was used routinely. The cutoff utilized as ≥10mg/L for diagnosis of "culture-confirmed early-onset sepsis." Based on when the CRP was obtained from the blood culture, which was taken at 3 days of birth, the results yielded varying specificities and sensitivities. If the CRP was obtained at ±4 hours, the sensitivity was 41.7%, specificity 89.9%, and positive likelihood ratio was 4.12. When obtained 24-72 hours later, the sensitivity became 89.5%, but specificity decreased to 55.7% and positive likelihood ratio to 2.02. During this time of routine CRP testing, there were higher rates of early-onset sepsis evaluation, antibiotic initiation, and antibiotic prolongation "in the absence of early-onset sepsis," but no difference in time to detection and in-hospital mortality with a period of non-routine CRP testing. The researchers ultimately concluded that the diagnostic performance of CRP in diagnosing early-onset sepsis was insufficient to warrant routine testing, as patient outcomes were not significantly affected with the elimination of routine CRP testing. Other factors with higher sensitivities, specificities, and positive likelihood ratios need to be included in the evaluation (Dhudasia et al., 2022).

IV. Guidelines and Recommendations

World Health Organization (WHO)

On May 16, 2018, the WHO released their first edition of the *Model List of Essential In Vitro Diagnostics* (EDL) "to advance universal health coverage, address health emergencies, and promote healthier populations." This list of in vitro diagnostics (IVD) is to be used as a reference of the essential diagnostic

tools for laboratories to complement their Model List of Essential Medicines. With respect to the diagnostic tool "to detect inflammation as an indicator of various conditions," the WHO recommends CRP either in an EIA (enzyme immunoassay) or RDT (rapid diagnostic test) assay format. The specimen type can be venous whole blood, serum, or plasma.

In 2019, the WHO released the *Second WHO Model List of Essential In Vitro Diagnostics.* In a table titled *General IVDs for Use in Clinical Laboratories*, CRP is once again listed. The WHO now recommends CRP in an RDT, latex agglutination assay or immunoassay format (WHO, 2019).

In 2020, the WHO released *The selection and use of essential in vitro diagnostics*, which included the third WHO model list. In the section on "General IVDs for community settings and health facilities without laboratories," the WHO performed an evaluation of utilizing ESR "to aid diagnosis and monitoring of certain infections and immune diseases; and as an alternative to a C-reactive protein (CRP) test where this is not available." In their table, they recommend using the Westergren assay format with sampling from venous whole blood. The WHO ultimately concluded that despite several guidelines recommending ESR to aid in diagnosing several inflammatory diseases, "there is no strong evidence supporting ESR as an essential test" since there are also high rates of false positives and false negatives. They conclude that CRP "should remain the preferred choice of test," except in cases of systemic lupus erythematosus and low-grade bone and joint infections since "there is evidence that the condition elevates ESR without causing a raise in CRP." As of this meeting, CRP now has the purpose "to monitor response to treatment" in addition to "detect inflammation as an indicator of various response conditions," and can be assayed as RDT, latex agglutination assay, and immunoassay with sampling venous whole blood, serum, and plasma (WHO, 2020).

National Comprehensive Cancer Network (NCCN)

In the guidelines concerning follow-up after completion of treatment up to five years, the NCCN (2022) recommends obtaining an interim history and physical "every 3-6 [months] for 1-2 [years], then every 6-12 [months] until year 3, then annually," as well as laboratory studies, which included a "[complete blood count], platelets, ESR if elevated at time of initial diagnosis, chemistry profile, as clinically indicated" with the same timeline. ESR is also used in determining the dosage of involved-site radiation therapy (ISRT). "A dose of 20 Gy following ABVD X 2 is sufficient if the patient has non-bulky stage I-IIA disease with an ESR <50, no extralymphatic lesions, and only one or two lymph node regions involved." An ESR ≥50 is considered as an "unfavorable risk factor" for stages I and II Hodgkin Lymphoma along with B symptoms. Please note that the NCCN guidelines concerning Hodgkin Lymphoma do not contain any information concerning the use of CRP as a diagnostic or prognostic tool (NCCN, 2022).

In the NCCN guidelines concerning the B-cell lymphomas under the section concerning Castleman Disease, the NCCN recommends (category 2A) as "essential" laboratory tests "LDH, CRP, [and] ESR." Within the discussion of the text, it does not mention if all three are required or if only a minimum of one of the three tests are essential in the workup. The guidelines for B-cell lymphomas do not list either CRP or ESR for follow-up testing post-treatment.

Regarding diagnostic criteria for idiopathic MCD (Multicentric Castleman Disease), minor diagnostic

criteria include elevated CRP (>10 mg/L) or ESR (>15 mm/h) where an "Evaluation of CRP is mandatory and tracking CRP levels is highly recommended, but ESR will be accepted if CRP is not available" (NCCN, 2023b).

In the NCCN guidelines concerning the T-cell lymphomas, they state that the "evaluation of serological markers such as rheumatoid factor (RF), antinuclear antibodies (ANA), and erythrocyte sedimentation rate (ESR) is useful in patients with autoimmune disease"(NCCN, 2023a). The guidelines concerning T-cell lymphomas do not mention the diagnostic or prognostic use of CRP.

In the NCCN guidelines concerning the T-cell lymphomas, they state that the "evaluation of serological markers such as rheumatoid factor (RF), antinuclear antibodies (ANA), and erythrocyte sedimentation rate (ESR) is useful in patients with autoimmune disease"(NCCN, 2020). [Please note that the Avalon policy AHS-G2022 covers ANA testing.] The guidelines concerning T-cell lymphomas do not mention the diagnostic or prognostic use of CRP.

American Society for Clinical Pathology (ASCP)

In the Choosing Wisely site of the ABIM Foundation, the ASCP released the recommendation to not "order an erythrocyte sedimentation rate (ESR) to look for inflammation in patients with undiagnosed conditions. Order a C-reactive protein (CRP) to detect acute phase inflammation" due to the sensitivity and specificity of CRP for acute phase of inflammation. "In the first 24 hours of a disease process, the CRP will be elevated, while the ESR may be normal. If the source of inflammation is removed, the CRP will return to normal within a day or so, while the ESR will remain elevated for several days until excess fibrinogen is removed from the serum." (ASCP, 2015)

European League Against Rheumatism (EULAR)

In 2009, EULAR issued their recommendations concerning the management of large vessel vasculitis. With a "Level of Evidence 3, Strength of recommendation C", they recommend "monitoring of therapy for large vessel vasculitis should be clinical and supported by measurement of inflammatory markers…. For patients with giant cell arteritis, a relapse is usually associated with a rise in ESR and CRP" (Mukhtyar et al., 2009). In this paper, no mention of the frequency of ESR and/or CRP testing is mentioned.

In 2013 in *EULAR recommendations for the use of imaging of the joints in the clinical management of rheumatoid arthritis* (Colebatch et al., 2013), they state that "baseline inflammatory disease measured by scintigraphy appears to be associated with radiographic progression. In addition, multiple regression analysis has demonstrated that progression of radiographic joint destruction was primarily predicted by ^{99m}Tc-IgG scintigraphy; joint swelling, ESR and IgM RF (Rheumatoid Factor) were not predictive. This suggests that scintigraphy may be superior to conventional clinical and laboratory measurements in the prediction of joint destruction." This set of guidelines did not include any mention concerning CRP or the frequency of ESR testing.

In 2015, EULAR and the American College of Rheumatology (ACR) issued joint recommendations concerning the management of polymyalgia rheumatica (PMR) (Dejaco et al., 2015). Within their recommendations, they list assessments that "every case of PMR should have…prior to the prescription

of therapy (primary or secondary care)." They include a basic laboratory workup "to exclude mimicking conditions and establish a baseline for monitoring of therapy", and they state that this includes "rheumatoid factor and/or anti-cyclic citrullinated peptide antibodies (ACPA), C-reactive protein and/or erythrocyte sedimentation rate (ESR), blood count, glucose, creatinine, liver function tests, bone profile (including calcium, alkaline phosphatase) and dipstick urinalysis." They do not state a specific preference of either CRP or ESR nor do they state the frequency of testing.

EULAR in 2016 updated their 2007 recommendations concerning the management of early arthritis (Combe et al., 2017). The 2016 updates included the following recommendation: "Monitoring of disease activity should include tender and swollen joint counts, patient and physician global assessments, ESR and CRP, usually by applying a composite measure. Arthritis activity should be assessed at 1-month to 3-month intervals until the treatment target has been reached." The recommendation concerning including both ESR and CRP did not change between the 2016 and 2007 recommendations. Within the discussion of the recommendations, they state, "In every patient with active arthritis, closely monitoring disease activity is now considered of particular importance in the therapeutic strategy to provide a good outcome…. Monitoring disease activity should be as frequent as the level of disease activity mandates, usually every 1-3 months, then potentially less frequently (such as every 6-12 months) once the treatment target has been achieved. Nevertheless, three changes were proposed to this item…. First, a composite measure was recommended as the method of choice to monitor disease activity; second, a specific time frame for monitoring structural damage was deliberately left out and third, patient-reported outcomes were expanded beyond functional assessments" (Combe et al., 2017).

In 2018, EULAR issued *EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice* (Dejaco et al., 2018). They make no recommendation concerning the preference of ESR or CRP nor do they state the frequency of testing; they do state "in patients with a high clinical suspicion of GCA (>50%), for example, in case of new-onset headache, visual symptoms, jaw claudication and elevated erythrocyte sedimentation rate (ESR) and C reactive protein, a positive ultrasound would result in a post-test probability of >95%."

American College of Rheumatology (ACR)

In 2012, ACR released their recommendations concerning the clinical practice of using disease activity measures of rheumatoid arthritis (RA) (Anderson et al., 2012). The recommend using the Disease Activity Score with 28-joint counts (DAS28), the Clinical Disease Activity Index, the Patient Activity Scale (PAS), the PAS-II, the Simplified Disease Activity Index (SDAI), and Routine Assessment of Patient Index Data with 3 measures. The DAS28 is a composite test that can use either CRP or ESR data. The ACR states that both the CRP or ESR used in the DAS28 have been validated in RA. Of the six activity measures recommended by the ACR, only DAS28 received "excellent" recommendations for all three psychometric properties—reliability, validity, and responsiveness. Within the guidelines, the ACR also issued the scores corresponding to remission, low/minimal, moderate, and high/severe RA for all of the disease activity measures, including the DAS28, as well as the mathematical formula using either CRP or ESR data to determine the DAS28. CRP is also used in the SDAI; however, the SDAI is rated as "good" for reliability because they state that "test-retest reliability for composite has not been evaluated" for the

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SDAI. No mention of frequency of testing is made. They do note that the "inclusion of acute-phase reactants in the DAS28 and SDAI complicates the logistics and timing using these measures in point-of-care clinical decision making. Although these measures have traditionally been used in clinical trials, academic medical centers, and large multispecialty clinics, logistical barriers have likely delayed their widespread adoption in smaller practice settings (Anderson et al., 2012)."

The ACR in 2015 (Singh et al., 2015) issued guidelines for the treatment of RA. While not specifying a preference of either CRP or ESR in diagnosing or predicting the prognosis of RA, they do state in their "Key provisos and principles" that "functional status assessment using a standardized, validated measure should be performed routinely for RA patients, at least once per year, but more frequently if disease is active." They also state that disease activity be measured using ACR-validated scales, including the aforementioned DAS28 and/or SDAI. Moreover, they define RA remission as "a tender joint count, swollen joint count, C-reactive protein level (mg/dl), and patient global assessment of ≤1 each or a Simplified DAS of ≤3.3, 1 of 6 ACR-endorsed disease activity measures".

Also, in 2015 (but published in 2016), the ACR and the Spondylitis Association of America (SAA) issued their joint recommendations concerning the treatment of ankylosing spondylitis (AS) and nonradiographic axial spondyloarthritis (Ward et al., 2016). Regarding "the treatment of patients with either active or stable AS…we conditionally recommend regular-interval use and monitoring of the CRP concentrations or erythrocyte sedimentation rate (ESR) over usual care without regular CRP or ESR monitoring." This received a "very low-quality evidence; vote 100% agreement" rating. They do make note that as of the time of publication "no studies addressed the effect of routine monitoring of a disease activity measure" but that "the panel thought that monitoring would be most helpful in patients with active symptoms as a guide to treatment." Testing is not required for every clinic visit. The two organizations reaffirm the same recommendations in their 2019 update (Ward et al., 2019).

In 2019, updated recommendations by the RA disease activity measures working group were published by England et al. (2019). Recommended tests include the Clinical Disease Activity Index (CDAI), the Simplified Disease Activity Index (SDAI), the Routine Assessment of Patient Index Data 3 (RAPID3), and the 28-Joint Disease Activity Score (DAS28). As noted above, the DAS28 is a composite test that can use either CRP or ESR data. The ACR states that both the CRP or ESR used in the DAS28 have been validated in RA. Updates to the management of rheumatoid arthritis were released by the ACR in 2022, but no mention of CRP or ESR were made (Arnold, 2022).

In 2021, the ACR published a guideline to provide evidence-based recommendations and expert guidance for the management of giant cell arteritis (GCA). They present 22 recommendations and 2 ungraded position statements for GCA and note that all but 1 of the recommendations are conditional due to very low- to low-quality evidence. They break these recommendations down into categories, including diagnostic testing, medical management, surgical intervention, and clinical/laboratory monitoring. All diagnostic recommendations involve biopsy or imaging- they do not recommend the use of CRP or ESR for diagnosis of GCA. However, they do recommend inflammation marker monitoring as part of clinical/laboratory monitoring. They define clinical monitoring as "assessing for clinical signs and symptoms of active disease, obtaining 4 extremity blood pressures, and obtaining clinical laboratory

results, including inflammation marker levels", with inflammation markers further defined as being CRP and ESR:

"Recommendation: For patients with GCA in apparent clinical remission, we strongly recommend long-term clinical monitoring over no clinical monitoring: The optimal frequency and length of monitoring are not well established and depend on factors including the duration of remission, site of involvement, risk of disease progression, whether the patient is receiving immunosuppressive therapy, and reliability of the patient to report new signs or symptoms. Clinical monitoring may include history taking, examinations, and laboratory and imaging studies. This is a strong recommendation given the minimal risks and potential catastrophic outcomes if monitoring is not performed.

Recommendation: For patients with GCA who have an increase in levels of inflammation markers alone, we conditionally recommend clinical observation and monitoring without escalation of immunosuppressive therapy. Increases in levels of inflammation markers such as erythrocyte sedimentation rate and C-reactive protein can be nonspecific (69). Therefore, increasing immunosuppressive therapy is not warranted in the setting of increased levels of inflammation markers in the absence of other signs of disease activity. However, these increased levels may warrant more frequent clinical and/or radiographic assessments for active disease" (Maz et al., 2021).

American Academy of Family Physicians (AAFP)

In 2013, the AAFP released *Recognition and Management of Polymyalgia Rheumatica and Giant Cell Arteritis*. For polymyalgia rheumatica (PMR), they note that "a normal ESR is found in 6% to 20% of persons with [PMR], although in those cases C-reactive protein level is elevated. ESR predicts relapse more reliably, but C-reactive protein is more sensitive, and is less affected by age and other factors." For giant cell arteritis (GCA), ESR is elevated in up to 89% of patients, but the sensitivity and specificity increase to 99% and 97%, respectively, if both ESR and CRP are tested. Regardless of using either ESR or CRP testing, the AAFP recommends that either ESR or CRP is tested at each clinic visit for patients with either PMR or GCA. (Caylor & Perkins, 2013)

American College of Radiology (ACR)

The ACR released their updated guidelines concerning the follow-up of Hodgkin lymphoma in 2014. They state that "limited data are available on the role of routine blood work in detecting relapses." ESR is listed as one of the tests conducted as routine blood work in follow-up of Hodgkin lymphoma. They summarize their findings as the following: "In general a majority of recurrences can be detected initially by history and physical examination rather than by routine imaging studies or blood tests such as ESR, CBC, and chemistry (Ha et al., 2014)." Four of the five variants they reviewed had ESR tests conducted 1 – 2 times per year, and the ACR rated the use of ESR as a 3, 5, 5, and 7 in these four variants where a "3" indicates "usually not appropriate," a "5" is "may be appropriate", and a "7" falls in the "usually appropriate" category.

The ACR released guidelines concerning management of multi-system inflammatory syndrome in children and devised a two-tier algorithm for diagnosis. ACR recommends routine lab tests as tier 1 testing, including complete blood count with manual differential, comprehensive metabolic panel, erythrocyte

sedimentation rate [ESR], CRP measurement, and testing for SARS-CoV-2 by polymerase chain reaction or serology. If tier 1 lab results include CRP ≥5 or ESR≥40 and one suggestive lab feature such as neutrophilia, lymphopenia, thrombocytopenia, hyponatremia, or hypoalbuminemia, the child should undergo tier 2 testing, which involves EKG and echocardiogram (Henderson et al., 2020; Henderson et al., 2021).

The British Society for Rheumatology (BSR) & British Health Professionals in Rheumatology (BHPR)

In 2010, BSR and BHPR issued joint guidelines concerning the management of giant cell arteritis (GCA) (Dasgupta, 2010; Dasgupta, Borg, Hassan, Alexander, et al., 2010). They recommend "early recognition and diagnosis of GCA is paramount. Particular attention should be paid to the predictive features of ischaemic neuro-ophthalmic complications." As part of this diagnostic recommendation, they specifically list laboratory tests that should be included— "full blood count, urea and electrolytes, liver function tests, CRP, ESR." They note that, although elevated ESR and CRP levels are hallmarks of GCA, "GCA can occur in the face of lower levels of inflammatory markers, if the clinical picture is typical." Another specific recommendation states, "Monitoring of therapy should be clinical and supported by the measurement of inflammatory markers (C; this is a consensus statement)" and that at each visit "full blood count, ESR/CRP, urea and electrolytes, [and] glucose" lab tests be performed.

Also, in 2010, BSR and BHPR issued joint guidelines concerning the management of polymyalgia rheumatica (PMR) (Dasgupta, Borg, Hassan, Barraclough, et al., 2010). For PMR, they recommend initial lab testing for diagnosis to include either ESR and/or CRP prior to initiating long-term steroid therapy. Also, during such therapy, they recommend monitoring either ESR or CRP every three months. This is a portion of the recommendation (B) of "vigilant monitoring of patients for response to treatment and disease activity."

The British Society for Rheumatology (BSR)

The BSR alone issued their guidelines for the management of systemic lupus erythematosus (SLE) in 2018 (Gordon et al., 2018). For the statement "CRP low or normal unless infection," the BSR gives an overall level of evidence of 2++ with a B grade of recommendation whereas they grade the statement "ESR correlates with active lupus" a 2+ and only a C grade of recommendation. "ESR is often raised in active SLE, but can also reflect persistent polyclonal hypergammaglobulinaemia, and is not a reliable marker of disease activity…. A significantly raised CRP is more likely to indicate infection, and patients with raised CRP will need therefore to be thoroughly screened for infection, given that infection is the commonest cause of death in lupus patients. In contrast, a raised ESR does not discriminate between active lupus and infection." They recommend that CRP is tested at initial diagnosis and then every 1-3 months during active disease states. Once stabilized, then testing frequency can be every 6-12 months. They also state that CRP testing should be conducted on mothers with SLE during pregnancy, but they do not state the frequency of the testing during pregnancy. This guideline is currently in revision.

The BSR has also published guidelines on the diagnosis and treatment of giant cell arteritis (GCA). Regarding which evaluations should be performed when starting treatment, the BSR states that "When starting glucocorticoids for suspected GCA, diagnostically relevant symptoms and signs should be

documented. Blood should be taken for full blood count, CRP and ESR before or immediately after commencing high-dose glucocorticoids. If GCA is strongly suspected, the first dose of glucocorticoid can be given without waiting for laboratory results (Mackie et al., 2020).” Further, the BSR provides a list of clinical assessments which should be carried out at or near a GCA diagnosis. These lists include “Measures of activity of GCA: laboratory markers of inflammation (CRP for all patients, plus either ESR or plasma viscosity) and full blood count (platelet count may be elevated in GCA).” Finally, regarding follow-up visits, “Each follow-up visit should include at least a full history, targeted physical examination and measurement of at least a full blood count, ESR and/or CRP, plus follow-up of any abnormalities relevant to the individual patient as well as drug-specific screening for toxicity (Mackie et al., 2020).” Revision for this guideline will be considered in 2024.

Canadian Rheumatology Association (CRA)

The 2012 guidelines by the CRA titled Canadian Rheumatology Association Recommendations for Pharmacological Management of Rheumatoid Arthritis with Traditional and Biologic Disease-modifying Antirheumatic Drugs recommends (with Level II and Strength B) “the presence of the following poor prognostic features should be assessed at baseline and considered when making treatment decisions: RF positivity, anti-CCP positivity, functional limitation, high number of swollen and tender joints, early erosions, extraarticular features, high ESR or CRP.” They also recommend (with Level I and Strength A) “RA care providers should monitor disease activity as frequently as every 1 to 3 months in patients with active RA.” The disease activity should be monitored by a validated method, such as DAS28 or SDAI. The most recent updated “living guidelines” for this statement does not include prognostic features or make recommendations for factors included in treatment decisions (Hazlewood et al., 2022).

In 2018, CRA released guidelines on assessment and monitoring of Systemic Lupus Erythematosus. Regarding diagnosis, CRA recommends that best clinical practice includes a complete history and physical examination at baseline with laboratory monitoring which could possibly include (but is not limited to) the following tests: “complete blood count (CBC), liver enzymes, creatine kinase, creatinine and estimated glomerular filtration rate (eGFR), urine routine/microscopic (urinalysis), urine protein-creatinine ratio, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complements (C3, C4), anti-dsDNA, antinuclear antibodies, antibodies to extractable nuclear antigens, antiphospholipid antibodies (aPL), lupus anticoagulant (LAC), anticardiolipin (aCL), anti-β2-glycoprotein I (anti-β2-GPI), and lipid profile. Follow up laboratory monitoring will depend on the patient’s clinical status and may include CBC, eGFR, urinalysis, urine protein-creatinine ratio, CRP, and/or ESR, C3, C4, and anti-dsDNA antibodies (Keeling et al., 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)."

National Institute for Health and Care Excellence (NICE)

NICE first issued the guidelines concerning irritable bowel syndrome (IBS) in 2008 with updates in 2015 and 2017. After initial assessment for IBS, they recommend ESR and CRP along with full blood count and antibody testing for celiac disease or tissue transglutaminase to exclude other possible diagnoses. They do not state anything concerning follow-up testing of either ESR or CRP. (NICE, 2017)

In 2020, NICE issues guidelines concerning management of rheumatoid arthritis (RA). In adults with active RA, they recommend measuring CRP and disease activity monthly in specialist care until remission or low disease activity is achieved (NICE, 2020).

American Academy of Orthopaedic Surgeons (AAOS)

The AAOS notes that "Strong evidence supports the use of [ESR and CRP] to aid in the preoperative diagnosis of prosthetic joint infection (PJI)." However, the AAOS remarks that neither biomarker is perfectly accurate for PJI diagnosis and should not be used as sole tests for diagnosis. Critically, neither marker informs clinicians of the microbiology of the PJI.

These guidelines were endorsed by IDSA, the American College of Radiology, and the Society of Nuclear Medicine and Molecular Imaging (AAOS, 2019).

Pediatric Infectious Diseases Society and the Infectious Diseases Society of America

In 2021, a guideline was released on the diagnosis and management of Acute Hematogenous Osteomyelitis (AHO) in pediatrics. In children with suspected AHO, they recommend performing a serum C-reactive protein (CRP) on initial evaluation. "Serum CRP has a low accuracy to establish the diagnosis of AHO, but in situations where AHO is confirmed, the serum CRP performed on initial evaluation can serve as the baseline value for sequential monitoring." They recommend against using serum PCT. In terms of ESR, they comment that the ESR is no longer used routinely to diagnose AHO in children. "ESR combined with CRP may slightly improve sensitivity and negative predictive value for the diagnosis of AHO, but specific thresholds and the overall clinical utility of using both CRP and ESR for diagnostic purposes remain uncertain" (Woods et al., 2021).

"There are no data to support a particular frequency of CRP monitoring during the course of AHO in children. Measurement every 2 to 3 days during the early therapeutic course, rather than daily, followed by weekly or other periodic measurement until normalization (or a clear trend toward normalization is evident) is an acceptable approach" (Woods et al., 2021).

V. Applicable State and Federal Regulations

Food and Drug Administration

Testing of serum acute phase reactants and ESR is performed in laboratories meeting Clinical Laboratory Improvement Act (CLIA) quality standards. 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). A search of the FDA Medical Devices database (FDA, 2018) on April 20, 2021, shows that the FDA has approved ESR systems from multiple companies, including the ESR Control -M Hematology Erythrocyte Sedimentation system (K972172) and the ESR Control -HC Hematology Erythrocyte Sedimentation system (K972170) by R & D Systems, the Seditainer Erythrocyte Sedimentation Rate System (K953994) from Becton Dickinson Vacutainer Systems, the Westergren Dispette for ESR (K831195) by Ulster Scientific, and the Dade ESR Kit (K823368) from American Dade.

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
85651	Sedimentation rate, erythrocyte; non-automated
85652	Sedimentation rate, erythrocyte; automated
86140	C-reactive protein
86141	C-reactive protein; high sensitivity (hsCRP)

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
8/24/22	Modified coverage criteria #1 and #2, and deleted coverage criteria #3 and #4.
10/27/23	The following changes were implemented: Addition of new coverage criteria #2: "For individuals without a diagnosed inflammatory condition, measurement of ESR **DOES NOT MEET COVERAGE CRITERIA**."; addition of "(conventional or high sensitivity)" to Note 1: "Coverage of ESR, CRP (conventional or high-sensitivity), or both CRP and ESR is designated based on the diagnosed or suspected inflammatory condition."; in Note 1, "Frequency of Testing" changed for AHO (from NS to "To confirm diagnosis; 2 to 3 days during the early therapeutic course; weekly until normalization (or a clear trend toward normalization is evident)), GCA (from at or near diagnosis to "to confirm diagnosis"), and RA (addition of "annually when disease is inactive").

G2155 General Inflammation Testing

Helicobacter pylori Testing

Policy #: AHS – G2044	**Prior Policy Name & Number (as applicable):**
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

Helicobacter pylori (*H. pylori*) is a spiral-shaped, gram negative bacteria that thrives while living in acidic environments, growing in close association with the stomach lining.H. *pylori* infection causes chronic inflammation (infection) in the stomach and is associated with conditions such as peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, and gastric mucosa associated lymphoid tissue (MALT) lymphoma (Lamont, 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 18 years of age and older, urea breath testing or stool antigen testing to diagnose an *H. pylori* infection **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) For individuals with dyspeptic symptoms.

   b) For individuals with active peptic ulcer disease (PUD).

   c) For individuals with past PUD without *H. Pylori* history.

   d) For individuals with low-grade gastric mucosa-associated lymphoid tissue (MALS) lymphoma.

   e) For individuals with a history of endoscopic resection of early gastric cancer (EGC).

   f) For individuals with gastric intestinal metaplasia (GIM).

   g) For individuals with uninvestigated dyspepsia who are under the age of 60 years without alarm features.

   h) For individuals initiating chronic treatment with a non-steroidal anti-inflammatory drug (NSAID).

   i) For individuals with unexplained iron deficiency anemia.

   j) For the evaluation of individuals with chronic immune thrombocytopenic purpura (ITP) and suspected *H. pylori* infection.

   k) For individuals with a family history of gastric cancer.

   l) For individuals who are first-generation immigrants from a high prevalence area.

2. For individuals 18 years of age and older, urea breath testing or stool antigen testing to measure the success of eradication of *H. pylori* infection (follow-up measurement of at least 4 weeks post-treatment) **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) For individuals with an *H. pylori*-associated ulcer.

   b) As part of the follow-up for individuals with persistent symptoms of dyspepsia following appropriate antibiotic treatment for *H. pylori*.

   c) For individuals with Gastric MALT Lymphoma.

   d) For individuals who have undergone resection of early gastric cancer.

3. For individuals 18 years of age and older undergoing endoscopic examination or who have alarm symptoms, a biopsy-based endoscopic histology test and either a rapid urease test or a culture with susceptibility testing to diagnose an H. pylori infection **MEETS COVERAGE CRITERIA**.

4. For individuals less than 18 years of age, urea breath testing or stool antigen testing to diagnose an H. pylori infection **MEETS COVERAGE CRITERIA** in any of the following situations:

a) For individuals with chronic ITP and suspected H. pylori infection.

b) To measure the success of eradication of H. pylori infection (follow-up measurement at least 4 weeks post-treatment).

5. For individuals less than 18 years of age, a biopsy-based endoscopic histology test and either a rapid urease test or a culture with susceptibility testing to diagnose an H. pylori infection **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) For individuals with gastric or duodenal ulcers.

   b) For individuals with refractory iron deficiency anemia (when other causes have been ruled out.)

6. Urea breath testing or stool antigen testing to diagnose an H. pylori infection **DOES NOT MEET COVERAGE CRITERIA** for any of the following situations:

   a) For asymptomatic individuals of all ages.

   b) For individuals 18 years and older with typical symptoms of gastroesophageal reflux disease (GERD) who do not have a history of peptic ulcer disease (PUD).

7. For individuals of all ages, serologic testing for *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA**.

8. For individuals less than 18 years of age, a biopsy-based endoscopic histology test and a rapid urease test or a culture with susceptibility testing to diagnose an *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:

   a) For children with functional abdominal pain.

   b) As part of an initial investigation in children with iron deficiency anemia.

   c) When investigating causes of short stature.

9. For individuals with recent use of antibiotics, proton pump inhibitors (PPIs), or bismuth, the urea breath test, stool antigen, or biopsy-based testing to diagnose an *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA**.

10. To diagnose an *H. pylori* infection, concurrent testing with any combination of the urea breath test, stool antigen testing, and/or biopsy-based testing **DOES NOT MEET COVERAGE CRITERIA**.

11. Nucleic acid testing for *H. pylori* **DOES NOT MEET COVERAGE CRITERIA**.

III. Scientific Background

Infection with *H. pylori* is common, with conservative estimates at 50% of the world's population affected. Prevalence in the United States is significant, estimated to be 30 – 40% in the general population (Siao & Somsouk, 2014). *H. pylori* is associated with many conditions, such as peptic ulcer disease, chronic gastritis, and gastric mucosa associated lymphoid tissue (MALT) lymphoma. Other conditions such as dyspepsia have been attributed to *H. pylori* as well (Lamont, 2022). Common symptoms of these conditions include gastritis, dyspepsia, heartburn, and stomach pain (Jensen, 2022; Longstreth, 2022).

Identification of *H. pylori* infection is accomplished with one or more of the several tests available. The choice of test is guided by the reason for the test, cost and availability of the test, the patient's age and clinical presentation, prevalence in a population, and the patient's use of certain medications. Testing for *H. pylori* infection is done for two main reasons; to detect an active infection that will be treated and to confirm eradication of the infection post-treatment. Invasive and non-invasive approaches have been used. Endoscopy and collection of biopsy specimens for evaluation of *H. pylori* infection and early gastric cancer detection typically is done in older individuals and those with "alarm" symptoms, including bleeding, unexplained anemia, unexplained weight loss, progressing dysphagia, recurrent vomiting, a family history of gastrointestinal cancer, or a personal history of esophagogastric malignancy. Tissue samples can be tested for *H. pylori* via methods such as a rapid urease test, culture, or staining. Molecular methods include PCR and next-generation sequencing, and serological methods include ELISA, immunoassays, and dried blood spots. Other non-invasive methods include urea breath test and stool antigen test. Testing for eradication of infection may be performed with the same tests used for diagnosis (Lamont, 2022).

Analytical Validity

Non-invasive options for detection of active *H. pylori* infection include urea breath tests and stool antigen testing. The stool antigen test is an immunoassay that detects the presence of *H. pylori* in a stool sample. The test is reported to have greater than 90% sensitivity and specificity for detection of active *H. pylori* infection, and its use has been FDA cleared for all ages. This test may be used for initial diagnostic purposes and for post-treatment testing. Urea breath tests, which take advantage of the bacteria's urease activity, may also be used to detect active *H. pylori* infection. The patient ingests a solution containing either ^{13}C or ^{14}C labeled urea, after a set amount of time, the patient's breath is collected and analyzed for the presence of ^{13}C or ^{14}C labeled CO_2. If *H. pylori* is present, it will have metabolized the labeled urea and labeled CO_2 will be detected, thus indicating infection with *H. pylori*. This test takes approximately 15-20 minutes (Lamont, 2022).

ELISA-based serological tests are also available for detection of *H. pylori*. However, serological tests often need validation at the local level, which may not be practical in routine practice. Furthermore, serological tests do not distinguish between past and present infections. Serological tests also have a very low positive predictive value in populations with low or average prevalence, as the antibodies will

be detected even after an infection has been treated or naturally resolved. In these low-prevalence areas, a positive serological test is more likely to be a false positive (Lamont, 2022).

Other tests such as PCR-based tests are infrequently used. The PCR test, despite its high accuracy, is often too expensive for routine use. In fact, nested PCR tests have approached 100% sensitivity and 100% specificity for detection of *H. pylori* (Singh et al., 2008), but the test may not be widely available and may be of limited use due to high cost (Lamont, 2022; Patel et al., 2014). PCR tests have been used for diagnostic purposes as well as identifying genetic variants of the bacteria and pathogenic genes present in a patient. A variety of body fluids, such as stool and saliva, have been used in PCR tests for this bacterial species (Patel et al., 2014).

Some medications are known to inhibit the growth or urease activity of *H. pylori* and can cause a false negative *H. pylori* test result. Proton pump inhibitors, antibiotics, and bismuth-containing medications may decrease sensitivity of tests, thereby increasing rates of a false negative. Eradication testing is often done weeks after treatment is completed (Lamont, 2022).

Dechant et al. (2020) evaluated the accuracy of various rapid urease tests (RUTs) and compared it with histopathology results. No differences were detected in the sensitivity or specificity of the various RUTs and RUTs had comparable results to histology; however, in patients treated with proton pump inhibitors and antibiotics. RUTs seemed to be more sensitive compared to histology. Pohl, Keller, Bordier, and Wagner (2019) discuss the drawbacks of RUTs, including false negative test results if the bacterial load is less than 10^4 in the gastric biopsy and false positive test results with some urease positive bacteria, affecting the sensitivity and specificity of RUTs. Commercially available RUTs, such as HpFast, CLOTest, and HpOne, have reported specificities ranging from 95% to 100%, but their sensitivity is moderate (85% to 95%) (Pohl et al., 2019).

Hussein et al. (2021) compared the sensitivity, specificity, positive, and negative predictive values of invasive tests (RUT and gastric tissue culture) and noninvasive tests (^{14}C-Urea breath test (^{14}C-UBT), stool antigen test, and CagA-IgG serology) to the gold standard quantitative PCR (qPCR) tests for *H. pylori* in Iraq. One hundred and fifteen participants strongly suspected of *H. pylori* infection were tested. Overall, the prevalence rates ranged from 47.8% to 70.4% depending on the test method. "The ^{14}C-UBT showed the highest overall performance with 97.5% sensitivity, 97% specificity, and total accuracy of 97.3% followed by SAT, RUT, Cag-IgG, and culture method." SAT had a sensitivity of 95.0% and a specificity of 91.2%. RUT had a sensitivity of 93.8% and a specificity of 94.1%. CagA-IgG had a sensitivity of 75.3% and a specificity of 85.3%. Gastric tissue culture had a sensitivity of 67.9% and a specificity of 79.4%. The authors conclude that ^{14}C-UBT "may be recommended as first choice due to its higher performance compared to other methods" (Hussein et al., 2021). Hassan et al. (2021) compared the accuracy, specificity, and sensitivity of the stool antigen test and the urea breath test in 45 children who underwent osophagogastroduodenoscopy between 2013 and 2019 in Sulaymaniyah City, Iraq. Histopathological findings from biopsies were used as a confirmatory diagnosis tool. The authors found that "UBT has a statistical significant correlation with result of biopsy, also it is more accurate and more sensitive than SAT, but they share same positive predictive value and same specificity." The authors conclude that UBT is preferred over SAT in children above six years (Hassan et al., 2021).

Abdelmalek et al. (2022) evaluated the accuracy and utility assurance of *H. pylori* stool antigen lateral flow immunochromatography assay (HpSA-LFIA) in Egypt. The study used stool samples from 200 gastric

patients and compared HpSA-LFIA results to the monoclonal antibody-based ELISA kit. The authors report that HpSA-LFIA achieved sensitivity of 93.75%, specificity of 59.76%, a negative predictive value of 98.00%, positive predictive value of 31.25%, and accuracy of 65.31%. The authors conclude that "HpSA-LFIA was not accurate enough to be the sole test for diagnosis and needs other confirmatory tests in case of positive conditions" (Abdelmalek et al., 2022).

Clinical Utility and Validity

The stool antigen test has been shown to have strong accuracy. A meta-analysis by Gisbert et al. (2006) focusing on 2499 patients of 22 studies found the diagnostic test to have a sensitivity of 0.94 and a specificity of 0.97. The monoclonal version of the test was shown to be more sensitive than the polyclonal one (0.95 vs 0.83). The authors also evaluated the diagnostic test after eradication of the bacteria in 957 patients of 12 studies. The authors evaluated the antigen test at 0.93 sensitivity and 0.96 specificity post-eradication (Gisbert et al., 2006).

A new automated LIAISON® Meridian *H. pylori* SA assay, a chemiluminescent immunoassay that uses novel monoclonal antibodies for capture and detection of the *H. pylori* stool antigen, was evaluated for its clinical performance. Opekun et al. (2020) studied the utility of this assay on 277 patients who tested positive for H. pylori infection from an endoscopy. Comparing histology, culture, and rapid urease test results, the assay delivered a sensitivity of 95.5% and specificity of 97.6%. The authors conclude that LIAISON® "brings reliable noninvasive testing for *H. pylori* to the laboratory that is in very good agreement with the current, more invasive biopsy-based methods such as histology, culture, or rapid urease test" (Opekun et al., 2020).

The rapid in-office, monoclonal test is widely used and provides significant benefit in terms of availability and speed. However, a study using the test as a reference to compare against a new test found the in-office test to only have a 0.50 sensitivity and 0.96 specificity out of 162 patients (Korkmaz et al., 2015).

The UBT has also been well-validated. A meta-analysis by Ferwana et al. (2015) including 3999 patients of 23 studies found the diagnostic test to have a pooled sensitivity of 0.96 and a pooled specificity of 0.93. The authors noted that their populations had significant heterogeneity but concluded that the UBT had high diagnostic accuracy for detecting an *H. pylori* infection (Ferwana et al., 2015). This test is often considered the gold standard for diagnosing an *H. pylori* infection (Patel et al., 2014).

Serological tests to assess infection have also been used. A meta-analysis by Loy et al. focused on commercial serological kits assessing *H. pylori*. Loy et al. found these kits to have a pooled sensitivity of 0.85 and specificity of 0.79. The authors concluded that there was no major difference in accuracy between any of the kits tested (Loy et al., 1996).

As costs of sequencing decreases, use of Next Generation Sequencing (NGS) to detect *H. pylori* infection and its antibiotic resistance has increased. In a study by Nezami et al. (2019), 133 H. *pylori* positive specimens from histological evaluation were analyzed by NGS to detect mutations in gyrA, 23S rRNA, and 16s rRNA genes. NGS detected H. *pylori* in 126/133 cases (95% sensitivity). NGS also detected multiple mutations associated with resistance in 92 cases (73%), one mutation in 63 cases (50%), and mutations in several genes in 29 cases (23%). In the 58 cases where treatment history was available,

therapy failure was observed in cases where the number of mutated genes was high. Therapy failed in 11/16 cases with multiple gene mutations and 5/27 cases with one gene mutation (Nezami et al., 2019).

Yang et al. (2019) performed a meta-analysis investigating the association between *H. pylori* and colorectal cancer. Twenty-seven studies encompassing 14357 cases were included. The authors found an increased rate of colorectal cancer with *H. pylori* infection (odds ratio [OR] = 1.27). The authors also identified odds ratios for certain subgroups, such as Western countries (OR = 1.34), serological testing (OR = 1.20), multiple methods of testing (OR = 2.63), and cross-sectional studies (OR = 1.92) (Yang et al., 2019).

Wang et al. (2019) performed a meta-analysis assessing the association between *H. pylori* and osteoporosis. 21 studies totaling 9655 patients were analyzed. The authors found that *H. pylori* infection was associated with an increased risk of osteoporosis with an odds ratio of 1.39. However, the decrease of bone mineral density in *H. pylori* positive patients was not found to be significant compared to *H. pylori* negative patients (Wang et al., 2019).

Zhou et al. investigated the association between *H. pylori* infection and non-alcoholic fatty liver disease (NAFLD). 15 studies including 97228 patients were evaluated. The authors identified an increased risk of NAFLD in *H. pylori* positive patients compared to *H. pylori* negative patients by an odds ratio of 1.19. Similar results were found despite differing subgroups, such as geographical locations. Testing method did not significantly change the results, and there was no significant difference when using multiple detection methods (Zhou et al., 2019).

Halland et al. (2021) assessed two novel enzyme assays (EIA), H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™, for the detection of *H. pylori* antigen in stool from 271 patients in America, Germany, and Bangladesh. The EIA results were compared to clinical diagnosis, which included histological analysis and rapid urease test. H. PYLORI QUIK CHEK™ had a sensitivity of 92% and a specificity of 91%. H. PYLORI CHEK™ had a sensitivity of 91% and a specificity of 100%. The authors concluded that "the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ assays demonstrate excellent clinical performance compared the composite reference method" (Halland et al., 2021). Rolon et al. (2021) have developed and tested a real-time PCR assay to simultaneously detect *H. pylori* infection and genotypic markers of clarithromycin resistance. *H. pylori* infection can be treated with clarithromycin-based therapy; The American College of Gastroenterology (ACG) recommends clarithromycin-based triple therapy as first-line treatment in regions where clarithromycin resistance is known to be below 15% in patients with no history of macrolide exposure. "Clarithromycin resistance is most commonly caused by point mutations in the 23S rRNA (rRNA) gene, including A2143G, A2142G, and A2142C, which result in decreased macrolide binding to the 23S rRNA ribosomal subunit; clarithromycin resistance is considered the main cause of clarithromycin therapy failure." The authors tested 524 stool samples. *H. pylori* stool antigen tests were used as a control test for *H. pylori* detection. Sanger sequencing was used as control tests for genetic susceptibility. PCR results were positive for 98% of positive antigen stool tests. "The clarithromycin-based triple therapy success was lower when resistance was predicted by PCR (41%) than when no resistance was predicted (70%; P = 0.03)." The authors conclude that the PCR assay can diagnose *H. pylori* infection and provide genetic susceptibility information. The authors suggest the need for susceptibility-guided therapy when clarithromycin-based therapy is considered (Marrero Rolon et al., 2022).

IV. Guidelines and Recommendations

American Gastroenterological Association (AGA)

The AGA recommends that "patients 55 years or younger without alarm features should receive *H. pylori* test and treat followed by acid suppression if symptoms remain" and note that "*H. pylori* testing is optimally performed by a 13C-urea breath test or stool antigen test." Alarm features include symptoms such as recurrent vomiting and weight loss. Additionally, the AGA indicates that "although the yield of endoscopy is low, it is recommended for patients older than 55 years of age and for younger patients…presenting with new-onset dyspepsia." They reason that endoscopy with biopsy is the preferred test for this age group because upper gastrointestinal malignancy becomes more common after age 55 years (Talley, 2005).

In 2015, the AGA published a technical review on Upper Gastrointestinal biopsy to evaluate dyspepsia in the absence of visible mucosal lesions and found that:

- In the defined population, biopsy of normal-appearing gastric mucosa can detect HP [*H. pylori*] infection that would be missed on the exam without biopsies. The quality of evidence is very low, and there are noninvasive methods to detect HP infection.
- "Detection of HP infection with tissue biopsy and its eradication in patients with dyspepsia is associated with symptom improvement and reduction of risk for HP-related comorbidities, including gastric cancer compared with no biopsy (or no eradication). The quality of evidence is moderate. The effect on symptom resolution is not universal and it does not appear to improve well-being. Quality of evidence for this statement is low" (Allen et al., 2015).

The AGA also released guidelines focusing on gastric intestinal metaplasia. In it, they recommend testing for *H. pylori* (followed by eradication) over no testing and eradication (Gupta et al., 2020).

The AGA released guidelines on gastrointestinal evaluation of iron deficiency anemia. AGA recommends that patients with iron deficiency anemia, without other identifiable etiology after bidirectional endoscopy, should undergo noninvasive testing for H. *pylori* over no testing at all to reduce the incidence of gastric cancer (Ko et al., 2020).

American College of Gastroenterology (ACG)/Canadian Association of Gastroenterology (CAG)

The ACG and CAG have released guidelines on testing for *H. pylori*:

- All patients with active peptic ulcer disease (PUD), a past history of PUD (unless previous cure of *H. pylori* infection has been documented), low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma, or a history of endoscopic resection of early gastric cancer (EGC) should be tested for *H. pylori* infection. Those who test positive should be offered treatment for the infection.
- In patients with uninvestigated dyspepsia who are under the age of 60 years and without alarm features, non-endoscopic testing for *H. pylori* infection is a consideration. Those who test positive should be offered eradication therapy.
- When upper endoscopy is undertaken in patients with dyspepsia, gastric biopsies should be taken to evaluate for *H. pylori* infection. Infected patients should be offered eradication therapy.

- Patients with typical symptoms of gastroesophageal reflux disease (GERD) without history of PUD need not be tested for *H. pylori* infection. For those who are found to be infected, treatment should be offered, acknowledging that effects on GERD symptoms are unpredictable.
- In patients taking long-term low-dose aspirin, testing for *H. pylori* infection could be considered.
- Patients initiating chronic treatment with a non-steroidal anti-inflammatory drug (NSAID) should be tested for *H. pylori* infection. Those who test positive should be offered eradication therapy.
- Patients with unexplained iron deficiency (ID) anemia despite an appropriate evaluation or idiopathic thrombocytopenic purpura should be tested for *H. pylori* infection.
- There is insufficient evidence to support routine testing and treating of *H. pylori* in asymptomatic individuals with a family history of "gastric cancer or patients with lymphocytic gastritis, hyperplastic gastric polyps and hyperemesis gravidarum".
- The ACG recommends the breath test and fecal stool antigen test as eradication tests, supported by moderate evidence (Chey et al., 2017).

Another set of joint guidelines from the ACG and Canadian Association of Gastroenterology (CAG) noted that dyspepsia patients under 60 should be tested for *H. pylori* (Moayyedi et al., 2017).

National Institute for Health and Care Excellence (NICE)

NICE recommends testing for *H. pylori* with a carbon-13 urea breath test or a stool antigen test. A re-test should be with a breath test. Office-based serological tests are not recommended. NICE recommends a "2-week washout period after proton pump inhibitor (PPI) use before testing for *Helicobacter pylori.*" NICE recommends that individuals with positive *H. pylori* tests be offered therapy to eradicate the bacteria; however, they note that re-testing to confirm eradication should not be routinely offered. NICE limits the recommendation for post-treatment testing to **"**people with peptic ulcer (gastric or duodenal)…6 to 8 weeks after beginning treatment, depending on the size of the lesion (NICE, 2019).

NICE released further guidelines in 2015 reaffirming the carbon-13 urea breath test and the stool antigen test to test for *H. pylori*. A locally validated lab-based serology test may also be used to assess *H. pylori*. NICE reaffirms the 2 week washout period before testing for *H. pylori* if the patient is on PPIs as well as the 4 week washout period if the patient is on antibiotics (NICE, 2015).

American College of Cardiology

The American College of Cardiology recommends testing for and eradicating *H. pylori* in patients with a history of ulcer disease before starting chronic antiplatelet therapy (Bhatt et al., 2008).

World Gastroenterology Organization

The World Gastroenterology Organization Global Guidelines on *Helicobacter pylori* recommends testing for *H. pylori* based on evidence-based indications, noting that these indications may differ in different regions of the world based on prevalence, resources, competing needs, and individual patient factors. The guidelines state that "peptic ulcer disease is the prime indication in most of the world." The guidelines list other indications for the treatment of *H. pylori* as: past or present duodenal and/or gastric ulcer, gastric MALT lymphoma, gastric mucosal atrophy and/or intestinal metaplasia, resection of gastric cancer, first-

degree relatives with gastric cancer, functional dyspepsia, NSAID use, before long-term aspirin therapy in patients at high risk of ulcers and ulcer-related complications, during long-term low-dose aspirin therapy in patients with a history of upper gastrointestinal bleeding and perforation, patients with gastroesophageal reflux disease who require long-term proton-pump inhibitors, as a strategy for gastric cancer prevention in communities with a high incidence, unexplained iron-deficiency anemia or idiopathic thrombocytopenic purpura, and patients' wishes after a full consultation with their physician (Katelaris et al., 2021).

European Association for Gastroenterology, Endoscopy and Nutrition (EAGEN), European Society of Neurogastroenterology and Motility (ESNM), and European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)

The pan-European guideline recommends the use of ^{13}C -urea breath tests as a noninvasive alternative for testing for "all indications of *Helicobacter pylori* testing if endoscopy is not required or if biopsies are contraindicated" and as "a preferred option for conformation of *Helicobacter pylori* eradication in adults and children." Alternatively, when there is indication for endoscopy and no contraindication for biopsy, the guidelines recommend RUT as the first-line diagnostic tests (Keller et al., 2021).

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

The ESPGHAN and NASPGHAN have issued updated guidelines for management of *H. pylori* in children and adolescents. They have proposed recommendations for diagnosis and management of *H. pylori* infection in pediatric patients. They have defined pediatric patients as children and adolescents below 18 years of age. The following recommendations were stated:

The guidelines recommend biopsies for rapid urease test and other cultures should only be taken if treatment is likely to be offered in the case of a confirmed infection. Treatment may be considered if *H. pylori* is an incidental finding at endoscopy.

The guidelines recommend against a "test and treat" strategy for *H. pylori* infection in children. The panelists explained that performing a noninvasive test to detect infection and treat is not needed because *H. pylori* infection usually does not cause any symptoms in the absence of peptic ulcer disease (PUD).

The guidelines recommend that "testing for *H. pylori* be performed in children with gastric or duodenal PUD."

The guidelines recommend against diagnostic testing for *H. pylori* infection in children with functional abdominal pain, iron deficiency anemia, and when investigating causes of short stature. Serology-based testing was also not recommended.

PPIs should be stopped two weeks before *H. pylori* testing, and antibiotics should be stopped four weeks before *H. pylori* testing. Diagnosis should be based on either: "positive culture or *H. pylori* gastritis on histopathology with at least 1 other positive biopsy-based test".

The non-invasive diagnostic testing was indicated in children when investigating causes of chronic immune thrombocytopenic purpura or for the assessment of anti-*H. pylori* therapy at least after 4 weeks of therapy (L. Jones et al., 2017).

Japanese Society for Pediatric Gastroenterology, Hepatology and Nutrition (JSPGHAN)

The JSPGHAN have updated their guidelines for *H. pylori* testing in pediatrics, including recommendations for diagnostic methods in children.

For diagnosis using endoscopic biopsy specimens, the guidelines recommend considering the performance and accuracy of the rapid urease test, recommending an additional urea breath test or stool antigen test when there is inconsistency between histopathology and the rapid urease test. The guidelines further recommend histological examination of gastric biopsies, and culture diagnostic tests to diagnose active *H. pylori* infection (Kato et al., 2020).

For diagnosis without endoscopic biopsy specimens, the guidelines recommend ^{13}C-urea breath test and stool antigen tests. To increase the diagnosis accuracy, the guidelines recommend more than two tests (two noninvasive tests or a biopsy-based and a noninvasive test) be completed. The guidelines recommend urea breath test or stool antigen test four or more weeks after treatment to confirm eradication of *H. pylori*, and recommend against using endoscopic biopsy methods and single serological tests to confirm eradication. The guidelines also recommend against anti-*H. pylori* antibody tests as a single test to diagnose *H. pylori* in a clinical setting (Kato et al., 2020).

Maastricht V/Florence Consensus Report

This report was published in 2017 on behalf of the European Helicobacter and Microbiota Study Group and Consensus panel. The panel reports that UBT is "the most investigated and best recommended non-invasive test in the context of a 'test-and-treat' strategy". The panel also notes that monoclonal tests can be used and that serological tests can be used only after validation. However, rapid "office" serology tests are not recommended and "should be avoided". The guidelines recommend the rapid urease test (RUT) as a first line diagnostic test if there is an indication for endoscopy and no contraindication for biopsy. The guideline state that *H. pylori* is linked to "unexplained iron deficiency anaemia (IDA), idiopathic thrombocytopenic purpura, and vitamin B12 deficiency", and in these disorders, an *H. pylori* infection should be "sought and eradicated." The guidelines state that PPIs should be stopped 2 weeks and antibiotics and other bismuth compounds should be stopped 4 weeks before testing for *H. pylori*. In cases of chronic (active) gastritis in which *H. pylori* is not detected by histochemistry, immunohistochemical testing of *H. pylori* can be used as an ancillary test. If histology is normal, no immunohistochemical staining should be performed. It is recommended to perform clarithromycin susceptibility testing when a standard clarithromycin-based treatment is considered as the first-line therapy, except in populations or regions with well documented low clarithromycin resistance (<15%). Pepsinogen (Pg) serology is considered the most useful non-invasive test to explore gastric mucosa status (non-atrophic vs atrophic). The PgI/PgII ratio can never be assumed as a biomarker of gastric neoplasia. UBT is the best option for confirmation of *H. pylori* eradication and monoclonal SAT is an alternative. It should be performed at least 4 weeks after completion of therapy (Malfertheiner et al., 2017).

The Maastricht IV from 2012 also addressed testing for the cagA and vacA variants, stating that no specific genetic or virulence markers can be recommended at this time (Malfertheiner et al., 2012).

American Society for Clinical Pathology (ASCP)

The ASCP recommends against using the serological tests for *H. pylori* and recommends the stool antigen and breath tests instead. The ASCP states that serological evaluation is no longer clinically useful and the stool and breath tests have superior statistical power (ASCP, 2016).

American Society of Hematology (ASH)

ASH published an update to the immune thrombocytopenic purpura guidelines in 2019. In it, they "suggest" that "Screening for *H pylori* be considered for patients with ITP in whom eradication therapy would be used if testing is positive". However, ASH still recommends against "routine testing for *H pylori* in children with chronic ITP" (Neunert et al., 2020).

Houston Consensus Conference

This conference included 11 experts on "management of adult and pediatric patients with *H. pylori*, from different geographic regions of the United States" and was convened to "discuss key factors in diagnosis of *H. pylori* infection, including identification of appropriate patients for testing, effects of antibiotic susceptibility on testing and treatment, appropriate methods for confirmation of infection and eradication, and relevant health system considerations". Two cohorts of approval were present: one of the 11 experts, and another consisting of a selected group of United States-based gastroenterologists. These recommendations were intended to provide practical advice for US practitioners, and also, guidelines to be adopted by US health care systems.

Recommendations approved by both groups are listed below:

- "Statement 1: We recommend that all patients with active *H pylori* infection be treated (100% agree/strongly agree, Grade 1A).
- Statement 2: All patients with current or past gastric or duodenal ulcers should be tested for *H pylori* infection (100% agree/strongly agree; Grade 1A).
- Statement 3: We recommend that all patients with uninvestigated dyspepsia be tested for *H pylori* infection (100% agree/strongly agree, Grade 1A).
- Statement 4: We recommend routine testing for *H pylori* infection in patients with reflux symptoms only if they are at high risk for *H pylori*-related disease (91% agree/strongly agree, Grade 1C).
- Statement 5: We recommend that patients with gastric mucosa-associated lymphoid tissue (MALT) lymphoma be tested for *H pylori* infection (100% agree/strongly agree, Grade 1B).
- Statement 6: We recommend that individuals with family history of gastric cancer be tested for *H pylori* infection (100% agree/strongly agree, Grade 1B).
- Statement 7: We recommend that patients who are first-generation immigrants from high prevalence areas be tested for *H pylori* infection (82% agree/strongly agree, Grade 1B).
- Statement 8: We suggest that patients of Latino and African American racial or ethnic groups may be considered for *H pylori* testing due to their high risk of infection (91% agree/strongly agree, Grade 2C)."

- Statement 17: We recommend that validated diagnostic testing of stool or gastric mucosal biopsy by culture and susceptibility, or molecular analysis be universally available (100% agree/strongly agree, Grade 1).
- Statement 18: We suggest that antibiotics that may be routinely evaluated for susceptibility include amoxicillin, clarithromycin, levofloxacin, metronidazole, and tetracycline (100% agree/strongly agree, Grade 2C).
- Statement 20: We recommend the use of tests for active *H pylori* infection (i.e., UBT, HpSAg testing) for the initial diagnosis (100% agree/strongly agree, Grade 1A).
- Statement 22: We recommend that serology not be utilized for detection of active *H pylori* infection (100% agree/strongly agree, Grade 1A).
- Statement 23: We recommend that bismuth and antibiotics be stopped at least 4 weeks before *H pylori* testing with tests for active infection (i.e., UBT, and HpSAg testing and histology; 100% agree/strongly agree, Grade 1C).
- Statement 27: We recommend that all patients receiving treatment for *H pylori* receive posttreatment confirmation of eradication. We recommend that only tests that evaluate for active infection, such as UBT, HpSAg test, or histology (if endoscopy is required for other reasons), are utilized for this purpose (100% agree/strongly agree, Grade 1A).
- Statement 28: Once appropriate testing has confirmed eradication, we recommend against further *H pylori* testing, (100% agree/strongly agree, Grade 1C)."

The following recommendations reached a consensus by the expert panel, but not the external group:

- "Statement 9: We recommend that patients with idiopathic thrombocytopenia be tested for *H pylori* infection (experts vs survey: 100% vs 68% agree/strongly agree, Expert Grade 1B).
- Statement 10: We suggest that patients receiving long-term PPIs (>1 month) be tested for *H pylori* infection (experts vs survey: 82% vs 68% agree/strongly agree, Expert Grade 2C).
- Statement 11: We recommend that family members residing in the same household of patients with proven active *H pylori* infections undergo *H pylori* testing (experts vs survey: 91% vs 78% agree/strongly agree, Expert Grade 1B).
- Statement 12: We recommend that individuals with a family history of peptic ulcer disease be tested for *H pylori* infection (experts vs survey: 91% vs (73% agree/strongly agree, Expert Grade 1B)" (El-Serag et al., 2018).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On Feb 22, 2012, the FDA approved the BreathTek UBT for *H. pylori* Kit created by Otsuka America Pharmaceutical, Inc. TheBreathTek UBT for *H. pylori* Kit (BreathTek UBT Kit) is intended for use in the qualitative detection of urease associated with *H. pylori* in the human stomach and is indicated as an aid in the initial diagnosis and post-treatment monitoring of *H. pylori* infection in adults, and pediatric patients 3 to 17 years old. The test may be used for monitoring treatment if used at 4 weeks following completion of therapy. The FDA notes its sensitivity and specificity to be 0.958 and 0.992 respectively (FDA, 2012).

On Jan 17, 2002, the FDA approved the BreathTek UBiT for *H. pylori* created by Meretek Diagnostics Inc. The scientific basis underlying the BreathTek UBT and the BreathTek UBiT UBT kit is identical. The urea breath test is FDA cleared for use in individuals 18 years of age and older (FDA, 2002).

On February 18, 2020, the FDA approved the PyloPlus UBT System created by ARJ Medical Inc. PyloPlus detects urease associated with *H. pylori* in the stomach and is indicated as an aid in the initial diagnosis of H. pylori infection in adults 18 years and older (FDA, 2023).

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
83009	Helicobacter *pylori*, blood test analysis for urease activity, non-radioactive isotope (e.g., C-13)
83013	Helicobacter *pylori*; breath test analysis for urease activity, non-radioactive isotope (e.g., C-13)
83014	Helicobacter *pylori*; drug admin
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step-method (e.g., reagent strip);
86677	Antibody; Helicobacter *pylori*
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87081	Culture, presumptive, pathogenic organisms, screening only
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (e.g., antibiotic gradient strip)
87186	Susceptibility studies, antimicrobial agent; microdilution or agar dilution (minimum inhibitory concentration [MIC] or breakpoint), each multi-antimicrobial, per plate
87205	Smear, primary source with interpretation; Gram or Giemsa stain for bacteria, fungi, or cell types
87338	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; Helicobacter pylori, stool
87339	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; Helicobacter pylori
88305	Level IV - Surgical pathology, gross and microscopic examination

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)
0008U	Helicobacter pylori detection and antibiotic resistance, DNA, 16S and 23S rRNA, gyrA, pbp1, rdxA and rpoB, next generation sequencing, formalin-fixed paraffin-embedded or fresh tissue, predictive, reported as positive or negative for resistance to clarithromycin, fluoroquinolones, metronidazole, amoxicillin, tetracycline and rifabutin
83009	Helicobacter *pylori*, blood test analysis for urease activity, non-radioactive isotope (e.g., C-13)
83013	Helicobacter *pylori*; breath test analysis for urease activity, non-radioactive isotope (e.g., C-13)
83014	Helicobacter *pylori*; drug admin
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step-method (e.g., reagent strip);

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
4/27/22	Modified wording throughout coverage criteria for clarity.

G2044 Helicobacter Pylori Testing

Diabetes Mellitus Testing

Policy #: AHS – G2006	**Prior Policy Name & Number (as applicable):** Hemoglobin A1c (AHS-G2006)
Implementation Date: 9/15/21	**Date of Last Revision:** 8/19/22, 10/16/23 (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

Diabetes describes several heterogeneous diseases in which various genetic and environmental factors can result in the progressive loss of β-cell mass and/or function that manifests clinically as hyperglycemia (Skyler et al., 2017).

Fasting plasma glucose (FPG) and oral glucose tolerance testing (OGTT) can be used in the diagnosis of diabetes mellitus. FPG is obtained from blood after a typically overnight period of not eating, whereas the OGTT is performed to understand an individual's response to a concentrated solution of glucose after 2 hours, typically in the setting of pregnancy (MayoClinic, 2022). In an asymptomatic individual, fasting plasma glucose ≥ 126 mg/dL or two-hour plasma glucose values of ≥200 mg/dL during a 75 g OGTT establish a diagnosis of diabetes. In reference to A1c values, a percentage ≥6.5% should raise clinical suspicion (Inzucchi & Lupsa, 2021). These assays are identified to be affordable alternatives to the more costly yet more convenient HbA1c level, and are more often used in the diagnosis of type 2 diabetes mellitus (Hayward & Selvin, 2022).

Glycated hemoglobin (A1c) results from post-translational attachment of glucose to the hemoglobin in red blood cells at a rate dependent upon the prevailing blood glucose concentration. Therefore, these levels correlate well with glycemic control over the previous eight to 12 weeks (Selvin, 2022). The measurement of hemoglobin A1c is recommended for diabetes management, including screening, diagnosis, and monitoring for diabetes and prediabetes.

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.

Diabetes Mellitus Testing, continued

1. For individuals with acute or persistent classic symptoms of diabetes mellitus, measurement of fasting plasma glucose (see Note 1) **MEETS COVERAGE CRITERIA.**
2. For individuals with a diagnosis of either Type 1 or Type 2 diabetes mellitus, measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA** in any of the following situations:
   a. Upon initial diagnosis to establish a baseline value and to determine treatment goals.
   b. Twice a year (every 6 months) in individuals who are meeting treatment goals and who, based on daily glucose monitoring, appear to have stable glycemic control.
   c. Quarterly in individuals who are not meeting treatment goals for glycemic control.
   d. Quarterly in individuals whose pharmacologic therapy has changed.
3. For prediabetic individuals, annual screening for type 2 diabetes with a fasting glucose test or measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA.**
4. Screening one every three years for prediabetes or Type 2 diabetes with a fasting plasma glucose test or measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA** for individuals with any of the following risk factors:
   a) For individuals who are overweight or obese.
   b) For first-degree relatives (see Note 2) of individuals with diabetes.
   c) For individuals with a history of cardiovascular disease.
   d) For individuals with hypertension.
   e) For individuals with hypercholesterolemia.
   f) For individuals with metabolic syndrome.
   g) For individuals who are obese and have acanthosis nigricans.
   h) For individuals with polycystic ovary syndrome.
   i) For individuals who were previously diagnosed with gestational diabetes mellitus (GDM).
5. For individuals who are positive for HIV, screening for diabetes and prediabetes with a fasting plasma glucose test **MEETS COVERAGE CRITERIA** in any of the following situations:
   a) For individuals starting antiretroviral therapy (ART).
   b) For individuals switching their ART.
   c) 3-6 months after starting or switching antiretroviral therapy.
   d) Annually when screening results were initially normal.
6. For individuals 10 years of age or older who have been diagnosed with cystic fibrosis (CF) but not with CF-related diabetes, annual screening for CF-related diabetes with an OGTT **MEETS COVERAGE CRITERIA.**

7. For overweight or obese children (after the onset of puberty or after 10 years of age, whichever occurs earlier), diabetes screening with a fasting plasma glucose test or an OGTT once every three years **MEETS COVERAGE CRITERIA** for individuals with any of the following risk factors:
    a) The individual has a maternal history of diabetes or gestational diabetes mellitus during the child's gestation.
    b) The individual has a family history of type 2 diabetes in first- or second-degree relatives (see Note 2).
    c) The individual has signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational-age birth weight).
8. For pregnant individuals, a fasting plasma glucose test or an OGTT up to once per month during pregnancy **MEETS COVERAGE CRITERIA**.
9. For individuals diagnosed with GDM during pregnancy, an OGTT **MEETS COVERAGE CRITERIA** in any of the following situations:
    a) To screen for persistent diabetes or prediabetes 4-12 weeks postpartum.
    b) For individuals with a positive initial postpartum screening result, repeat screening to confirm a diagnosis of persistent diabetes or prediabetes.
10. For all other situations not previously described (see Note 3), measurement of hemoglobin A1c **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: While this policy provides evidence-based reasons for fasting or random plasma glucose testing in the diagnosis of diabetes, these tests have clinical use outside the scope of this policy and thus are not restricted to the criteria detailed above. According to the American Diabetes Association (ADA), measurement of plasma glucose is sufficient to diagnose diabetes mellitus in a patient with classic symptoms (polyuria, polyphagia, polydipsia).

Note 2: 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.

Note 3: Measurement of hemoglobin A1c **should not** be performed in **any** of the following situations:

1) In pregnant individuals not already diagnosed with diabetes.
2) In individuals not already diagnosed with diabetes.
3) In conjunction with measurement of fructosamine.
4) In individuals with a condition associated with increased red blood cell turnover, such as sickle cell disease, hemodialysis, recent blood loss or transfusion, or erythropoietin therapy.

III. Scientific Background

Diabetes Mellitus Testing, continued

Diabetes is a major health concern in the United States. According to the Centers for Disease Control and Prevention:

- Prevalence: In 2019, 37.3 million Americans, or 11.3% of the population, had diabetes. Approximately 1.9 million American children and adults have type 1 diabetes, including about 244, 000 children and adolescents.
- Diagnosed and undiagnosed: Of the 37.3 million, 28.7 million were diagnosed, and 8.5 million were undiagnosed.
- Prevalence in seniors: The percentage of Americans age 65 and older remains high, at 29.2%, or 15.9 million seniors (diagnosed and undiagnosed).
- New cases: 1.4 million Americans are diagnosed with diabetes every year.
- Prediabetes: In 2019, 96 million Americans age 18 and older had prediabetes.
- Deaths: Diabetes remains the 7th leading cause of death in the United States in 2019, with 87,647 death certificates listing it as the underlying cause of death, and a total of 282,801 death certificates listing diabetes as a cause of death.
- Total economic cost of diabetes care in the United States: $327 billion in 2017 (ADA, 2017; CDC, 2020).

Diabetes can be classified into the following categories:

- "Type 1 diabetes (due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency)"
- "Type 2 diabetes (due to a progressive loss of β-cell insulin secretion frequently on the background of insulin resistance)"
- "Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation)"
- "Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation)" (ADA, 2021a).

The diagnosis of diabetes mellitus is easily established when a patient presents with classic symptoms of hyperglycemia, which include polyuria, polydipsia, nocturia, blurred vision, and, infrequently, weight loss. The frequency of symptomatic diabetes has been decreasing in parallel with improved efforts to diagnose diabetes earlier through screening. Increasingly, the majority of patients are asymptomatic, and hyperglycemia is noted on routine laboratory evaluation, prompting further testing (Inzucchi & Lupsa, 2021).

Glycated hemoglobin A1c (also known as HbA1c, A1c, glycohemoglobin, hemoglobin A1c) testing plays a key role in the management of diabetes. New hemoglobin enters circulation with minimal glucose attached. However, glucose irreversibly binds to hemoglobin based on the surrounding blood glucose concentration. Therefore, A1c is considered a measure of blood glucose level, albeit an indirect one. It is best correlated with the mean glucose level over the last 8 to 12 weeks as red blood cells experience

significant turnover. Various factors may affect the reliability of A1c (atypical hemoglobins or hemoglobinopathies, chronic kidney disease, et al.), but most assays have been standardized to the Diabetes Control and Complications Trial (DCCT) standard, which "estimated the mean blood glucose concentrations derived from seven measurements a day (before and 90 minutes after each of the three major meals, and before bedtime), performed once every three months and compared the average glucose concentration with A1c values in patients with type 1 diabetes" (Selvin, 2022).

The HbA1c assay provides information about the degree of long-term glucose control (Nathan et al., 1984), and has been recommended for the diagnosis and monitoring of diabetes (ADA, 2010; IEC, 2009). Various methods of HbA1c measurement include chromatography based HPLC assay, boronate affinity, antibody-based immunoassay, and enzyme based enzymatic assay (Kanyal Butola et al., 2021). Long-term blood sugar control has been associated with decreased risk of retinopathy, nephropathy, neuropathy, and cardiovascular disease, peripheral arterial, cerebrovascular disease (Hanssen et al., 1992) and myocardial fibrosis in adults with diabetes (Al-Badri et al., 2018). Higher HbA1c variability has been associated with higher all-cause mortality in patients with Type 2 Diabetes (Gu et al., 2018).

Fasting plasma glucose is a method of glucose monitoring that measures an individual's glucose level typically in a period defined with no caloric intake for eight hours or more. Its usage in the diagnosis of diabetes lies primarily in gestational diabetes, along with the oral glucose tolerance test, but HbA1c, fasting plasma glucose, or oral glucose tolerance tests with their respective positive results can be used in diagnosing diabetes mellitus in nonpregnant individuals as well. To diagnose diabetes in asymptomatic individuals, a fasting plasma glucose has to be ≥ 126 mg/dL. For diagnosing prediabetes, an individual may have "impaired fasting glucose," which would present with a range of 100-125 mg/dL (Hayward & Selvin, 2022; Inzucchi & Lupsa, 2021).

The oral glucose tolerance test (OGTT) can be more inconvenient and used in the setting to diagnose gestational diabetes mellitus (GDM). Normally, 75g of glucose is ingested by the patient, and if the patient has a two-hour plasma glucose value of ≥200 mg/dL, a diagnosis of diabetes can be made. The test can also be performed at one hour with 50g oral glucose, with positive GDM diagnostic results between 130-140 mg/dL as part of a two-step approach with the three-hour 100g test, which can be diagnostic of GDM with two elevated values. For prediabetes with an accompanied "impaired glucose tolerance," a two-hour plasma glucose value between 140-199 mg/dL is used. However, the WHO requires an additional FPG <126 in addition to the two hour plasma glucose value to establish impaired glucose tolerance (Durnwald, 2022; Hayward & Selvin, 2022).

Analytical Validity

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on HbA1c Standardization has developed a reference measurement system and the measurement of HbA1c is currently well-standardized (Hoelzel et al., 2004), and a sound reference system is in place to ensure continuity and stability of the analytical validity of HbA1c measurement (Weykamp et al., 2008). In contrast, plasma glucose concentration remains difficult to assay with consistent accuracy (Gambino, 2007). HbA1c has greater analytical stability (consistency with repetitive sample testing) and less day-to-day variability than either the fasting plasma glucose (FPG)or 2-h PG (Petersen et al., 2005; Rohlfing et al., 2002). For any given individual, the HbA1c exhibits little short-term biologic variability; its coefficient of variation (CV) is 3.6%, compared to FPG (CV of 5.7%) and 2-h PG (CV of 16.6%) (Malkani & Mordes, 2011; Selvin et al., 2007).

Diabetes Mellitus Testing, continued

A sample proficiency testing survey performed by the National Glycohemoglobin Standardization Program (NGSP) and College of American Pathologists (CAP) evaluated the accuracy of A1c assays. The survey found that "method-specific, between-laboratory CV's [*sic*] ranged from 0.9% to 4.5%" and "approximately 91% of laboratories are using methods with CVs <3.5% at all four HbA1c levels." The survey also noted the current pass limit was ±6%, but using a pass rate of 5%, 92.9% to 96.1% of labs passed (NGSP, 2019).

Clinical Utility and Validity

Testing A1c, FPG, and 2-h PG measure different aspects of glycemia and are frequently discordant for diagnosing diabetes. A1c ≥6.5% identifies fewer individuals as having diabetes than glucose-based criteria; however, a recent study concluded that 12% of patients can be misclassified with respect to diabetes diagnosis due to laboratory instrument error in measuring glucose (Miller et al., 2008). The New Hoorn Study analyzed the diagnostic properties of the A1c, using OGTT as the diagnostic criterion (van 't Riet et al., 2010). The analysis suggested that an A1c of 5.8% had a sensitivity of 72% and specificity of 91%. This compares with specificity of 24% and sensitivity of 99% for the A1c cut-point of 6.5%. On the other hand, the 6.5% cutpoint had a positive predictive value of 93%, compared with a positive predictive value of only 24% for a cut-point of 5.8% (Malkani & Mordes, 2011).

When using the reference diagnosis of diabetes being a two-hour blood glucose >200 mg/dL (11.1 mmol/L) during an OGTT, the specificity of FPG ≥126 mg/dL was >95% and sensitivity about 50%, with possibly lower sensitivities and specificities for individuals over 65 years (Blunt et al., 1991). With the same OGTT reference, the specificity and sensitivity of an A1c ≥6.5%, as per diagnosis of diabetes, were reported as 79% and 44%, respectively (Kramer et al., 2010).

Cowie et al. (2010) "examined prevalences of previously diagnosed diabetes and undiagnosed diabetes and high risk for diabetes using recently suggested A1c criteria in the U.S. during 2003–2006. We compared these prevalences to those in earlier surveys and those using glucose criteria." 14611 individuals were included (completed a household interview) and classified for diagnosed diabetes and by A1c, fasting, and 2-h glucose challenge values. Diagnostic values for A1c were ≥6.5% for "undiagnosed" diabetes and 6%-6.5% for "high risk" of diabetes. The authors found that by these A1c diagnostic values, the "crude prevalence" of diabetes in adults older than 20 years was 20.4 million, of which 19% went undiagnosed based on A1c ≥ 6.5%. The authors then stated that the A1c criteria only diagnosed 30% of the undiagnosed diabetic group (Cowie et al., 2010).

Mamtora et al. (2021) assessed the clinical utility of point of care (POC) HbA1c testing in the ophthalmology outpatient setting. Forty-nine patients with diabetic retinopathy underwent POC HbA1c testing and blood pressure measurement. Of the 49 patients, 81.6% had POC readings above the recommended HbA1c levels and only 16.3% of these patients were aware of their elevated HbA1c levels. 14 patients (33.3%) with high HbA1c readings were referred to secondary diabetic services and 88.8% of patients felt like the test was useful. The authors suggest that POC HbA1c testing is a "cost-effective, reproducible and clinically significant tool for the management of diabetes in an outpatient ophthalmology setting, allowing the rapid recognition of high-risk patients and appropriate referral to secondary diabetic services" (Mamtora et al., 2021).

Goodney et al. (2016) evaluated the consistency of A1c testing of diabetes patients and its effect on cardiovascular outcomes. The study included 1,574,415 Medicare patients with diabetes mellitus, and

the consistency of testing was separated into three categories: “low (testing in 0 or 1 of 3 years), medium (testing in 2 of 3 years), and high (testing in all 3 years).” 70.2% of patients received high-consistency testing, 17.6% received medium-consistency, and 12.2% received low-consistency. Major adverse cardiovascular events (MACE) included “death, myocardial infarction, stroke, amputation, or the need for leg revascularization”. Low-consistency patients was associated with death or other adverse events (hazard ratio: 1.21). The authors concluded that “consistent annual hemoglobin A1c testing is associated with fewer adverse cardiovascular outcomes in this observational cohort of Medicare patients of diabetes mellitus (Goodney et al., 2016).”

The GOAL study (Al Mansari et al., 2018) used A1c to assess diabetes control in a real-world practice study aimed to assess predictive factors for achieving the glycemic hemoglobin A1c (HbA1c) at 6 months as targeted by the treating physician in adults with type 2 diabetes. 2704 patients with a mean A1c of 9.7% were enrolled. After 6 months, lower baseline A1c (≥ 8.5% vs <7%) was found to be a predictive factor for achieving glycemic control. The authors also observed “absolute changes in the mean HbA1c of −1.7% and −2% were observed from baseline to 6 and 12 months, respectively (Al Mansari et al., 2018).”

Mitsios et al. (2018) evaluated the association between A1c and stroke risk. 29 studies (n=532779) were included. The authors compared the non-diabetic A1c range (<5.7%) to the diabetic range (≥6.5%) and found that the diabetic range was associated with a 2.15-fold increased risk of first-ever stroke. The pre-diabetes range of 5.7%-6.5% was also not associated with first-ever stroke. The authors also observed that for every 1% increase in A1c, the hazard ratio of first-ever stroke increased (1.12-fold for non-diabetic ranges, 1.17 for diabetic ones). This increased risk was also seen for ischemic stroke, with a hazard ratio of 1.49 for non-diabetic ranges and 1.24 for diabetic ranges (Mitsios, Ekinci, Mitsios, Churilov, & Thijs, 2018).

Ludvigsson et al. (2019) evaluated the association between preterm birth risk and periconceptional HbA1c levels in women with type 1 diabetes (T1D). Preterm birth was defined as <37 weeks and several secondary outcomes were also examined, which were “neonatal death, large for gestational age, macrosomia, infant birth injury, hypoglycemia, respiratory distress, 5-minute Apgar score less than 7, and stillbirth”. A total of 2474 singletons born to women with T1D and 1165216 reference infants (children born to mothers without T1D) were included. The authors identified 552 preterm births in the T1D cohort (22.3%) compared to 54287 in the control cohort (4.7%). Incidences of preterm birth were measured at several separate thresholds, including <6.5%, 6.5%-7.8%, 7.8%-9.1%, and >9.1%. The T1D cohort’s adjusted risk ratios (aRR) of preterm birth compared to the control cohort were as follows: 2.83 for <6.5%, 4.22 for 6.5%-7.8%, 5.56 for 7.8%-9.1%, and 6.91 for >9.1%. The corresponding aRRs for “medically indicated preterm birth” (n=320) were 5.26, 7.42, 11.75 and 17.51 respectively. Increased HbA1c levels were also found to be associated with the secondary clinical outcomes. The authors concluded that “the risk for preterm birth was strongly linked to periconceptional HbA1c levels. (Ludvigsson et al., 2019)”

Saito et al. (2019) examined the association of HbA1c variability (defined as visit-to-visit) and later onset of malignancies. The authors included 2640 patients 50 years or older, with diabetes. A total of 330 patients (12.5%) developed malignancies during follow-up. The authors stratified the patients into quartiles of glycemic variability (defined as standard deviation of HbA1c) and found a “dose-dependent association with tumorigenesis” in the three highest quartiles. The odds ratios were as follows: 1.20 for the second quartile, 1.43 for the third, and 2.19 for the highest. The authors concluded that “these

results demonstrated that visit-to-visit HbA1c variability is a potential risk factor for later tumorigenesis. The association may be mediated by oxidative stress or hormone variability. (Saito et al., 2019)"

Mañe et al. (2019) evaluated the "suitability of first-trimester fasting plasma glucose and HbA1c levels in non-diabetic range to identify women without diabetes at increased pregnancy risk". Primary outcomes were defined as "macrosomia and pre-eclampsia" and secondary outcomes were defined as "preterm delivery, Caesarean section and large-for-gestational age". A total of 1228 pregnancies were included. Women with an HbA1c of ≥5.8% were found to have an increased risk of marcosomia (odds ratio [OR] = 2.69), an HbA1c of ≥5.9% was found to be associated with a three-fold risk of pre-eclampsia, and an HbA1c of ≥6% was found to be associated with a four-fold risk of "large-for-gestational age". Fasting plasma glucose levels were not found to be associated with any pregnancy outcome. (Mañé et al., 2019).

Arbiol-Roca et al. (2021) studied the clinical utility of HbA1c testing as a biomarker for detecting gestational diabetes mellitus (GDM) and as a screening test to avoid the use of the oral glucose tolerance test (OGTT). HbA1c levels were measured in 745 pregnant women and GDM was diagnosed in 38 patients based on HbA1c, age, and BMI. A cut off HbA1c value of 4.6% was determined to decide whether OGTT was needed or if it could be avoided. Using 4.6% HbA1c as the cut off value prevented two false negatives, but only decreased the number of OGTTs performed by 7.2%. The authors conclude that "adoption of HbA1c as a screening test for GDM may eliminate the need of OGTT." Although the HbA1c test does not have sufficient sensitivity and specificity to be used as the sole diagnostic test, " the use of a rule-out strategy in combination with the OGTT could be useful" (Arbiol-Roca et al., 2021).

However, the use of hemoglobin A1c testing is not useful in predicting all forms of dysglycemia. Tommerdahl et al. (2019) evaluated several biomarkers for their accuracy in screening for cystic fibrosis (CF)-related diabetes. These biomarkers included "hemoglobin A1c (HbA1c), 1,5-anhydroglucitol (1,5AG), fructosamine (FA), and glycated albumin (GA)" and were compared to the current gold standard, OGTT 2-hour glucose. Fifty-eight patients with CF were included and "area under the receiver operative characteristic (ROC-AUC) curves were generated." All ROC-AUCs for each biomarker were "low" both for cystic fibrosis-related prediabetes (CFPD, ROC-AUC 0.52-0.67) and CF-related diabetes (CFRD) (0.56-0.61). For CFRD, HbA1c was measured to have a 78% sensitivity and 41% specificity at a cutoff of 5.5%, which corresponds to a ROC-AUC of 0.61. The authors concluded that "All alternate markers tested demonstrate poor diagnostic accuracy for identifying CFRD by 2hG (Tommerdahl et al., 2019)."

In a retrospective review of the UMass Memorial Health System electronic medical records from between 1997 and 2019, Darukhanavala et al. (2021) evaluated the appropriateness of HbA1c as a screening tool for identifying patients with pre-CFRD (cystic fibrosis-related diabetes) dysglycemia to minimize the burden of annual two-hour oral glucose tolerance tests (OGTTs). The study included 56 patients categorized according to OGTT results (American Diabetes Association criteria): normal glucose tolerance (n=34), indeterminant glycemia (INDET, n=6), impaired fasting glucose (IFG, n=7), or impaired glucose tolerance (IGT, n=9). It was found that HbA1c was positively correlated with blood glucose levels at the various time cut-points (hour 0, hour 1, and hour 2), though the associations were quite weak (r = 0.248, r = 0.219, and r = 0.369, respectively). Furthermore, t-tests conducted suggested that the mean HbA1c was not significantly different between patients with normal glucose tolerance and those in the INDET (p = 0.987), IFG (p = 0.690), and IGT (p = 0.874) groups, confirmed by ANOVA (p = 0.250). Consequently, the

authors reported that the "results do not support the use of HbA1c as a possible screening tool for pre-CFRD dysglycemic states, specifically INDET, IFG, and IGT" (Darukhanavala et al., 2021).

By combining administrative datasets from the Veterans Health Administration and Medicare, Zhao et al. (2021) evaluated the impact of hemoglobin A1c (A1c) variability—the coefficient of variation, described by A1c standard deviation divided by the average A1c value overall and expressed as a percent—on the risk of hypoglycemia-related hospitalization (HRH) in veterans with diabetes mellitus. In this study sample of 342059 patients, the authors identified a "consistent and positive relationship between A1c variability and HRH" and noted that "Average A1c levels were also significantly and independently associated with HRH, with levels <7.0% (53 mmol/mol) associated with lower risk and levels >9% (75 mmol/mol) conferring greater risk". Due to these different levels of variability all remaining strong predictors of HRH risk up to three years following the baseline period, authors concluded that "tracking A1c levels alone may be insufficient to mitigate risk". It was also acknowledged that a few limitations affected the generalizability of the study, such as the lack of socioeconomic data, the study sample being predominantly white males, and including only veterans, the latter of which is a population where comorbidities are more prevalent. Consequently, these data may be reflective of "the complex interplay of disease severity, treatment, and sociodemographic factors", as is the case with other clinical findings (Zhao et al., 2021).

While poor outcomes of coronavirus disease 2019 (COVID-19) have been linked to diabetes, its relation to pre-infection glycemic control is still unclear. Because of this, Merzon et al. (2021) investigated the association between pre-infection HemoglobinA1c (A1C) levels and COVID-19 severity as assessed by need for hospitalization in a cohort of 2068 patients (ages 14 to 103) with diabetes tested for COVID-19 in Leumit Health Services, Israel, between February 1 and April 30, 2020. Of the patients in this cohort, 183 (8.85%) were diagnosed with COVID-19. A comparison of the mean HbA1c of those who were COVID-19 positive (7.19%, 95% CI: 6.81%-7.57%) and the mean of those who were COVID-19 negative (6.59%, 95% CI: 6.52%-6.65%) was found to be statistically significant ($p<0.05$). The authors expounded further by reporting the clinical characteristics of patients with diabetes hospitalized due to COVID-19 by demonstrating that the mean Hb1Ac levels between those hospitalized (n=46, 7.75%, 95% CI: 7.17%-8.32%) and those not hospitalized (n=137, 6.83%, 95% CI: 6.54%-7.13%) were also statistically significant ($p<0.005$). Additionally, "In a multivariate logistic regression model adjusting for multiple potential risk factors and chronic conditions which may have a deleterious effect on disease outcomes (including age, sex, smoking, IHD, SES, depression/anxiety, schizophrenia, dementia, hypertension, CVA, CHF, chronic lung disease, and obesity), only HbA1c ≥ 9% remained a significant predictor for hospitalization." Given the evidence, the researchers urge "Paying special attention to patients with diabetes and an HbA1c ≥ 9 while allowing a more lenient approach to patients with well controlled disease", as this can reduce economic, social, and patient burden, especially for those who are at the greatest risk for reacting severely to COVID-19 (Merzon et al., 2021).

Xie et al. (2021) investigated the role of FPG and glucose fluctuation on the prognosis of COVID-19 patients who already had prior diagnoses of diabetes. Through a multivariate Cox analysis, the researchers found that FPG was "an independent prognostic factor of overall survival after adjustment for age, sex, diabetes, and severity of COVID-19 at admission (HR: 1.15, 95% CI: 1.06-1.25)." However, blood glucose fluctuation was associated with COVID-19 disease progression, as proven by the results found from the indices of the standard deviation of blood glucose and the largest amplitude of glycemic excursions. Both FPG and blood

glucose fluctuation indices were also found to be positively associated with increased presence of inflammatory markers associated with COVID-19, such as the "white blood cell absolute count, neutrophil count, C-reactive protein (CRP), alkaline phosphatase, a-hydroxybutyrate dehydrogenase (α-hbdh), gamma-glutamyl transferase (GGT), lactate dehydrogenase, [and] D-dimer." Ultimately, it was concluded that diabetes was not an independent risk factor for in-hospital death of COVID-19 patients, as these findings were identified regardless of diabetes status (Xie et al., 2021).

Yang et al. (2019) aimed to find the appropriate threshold for fasting plasma glucose for defining prediabetes among children and adolescents. The sample was selected from school-aged children in Taiwan via a nationwide survey administered between 1992-2000, who then underwent physical examinations and blood tests if they exhibited abnormal urine test findings. The researchers found that the incidence of pediatric diabetes increased with increasing fasting plasma glucose levels, and those with FPG > 5.6mmol/L had higher adjusted hazard ratios. Additionally, "the association between fasting plasma glucose and incident pediatric diabetes and the area under the receiver-operating characteristic curve were similar in boys and girls and were higher in the age group 12-18 years." In using 4.75 mmol/L as the optimal threshold for children 6-11 years, the sensitivity was 65% and specificity was 51%. For the threshold of 5.19 mmol/L among children 12-18 years, the sensitivity was 60% and the specificity was 73%. This supports utilizing FPG as a supplement for diagnosing prediabetes among pediatric patients, which may contribute to better disease management.

Geifman-Holtzman et al. (2010) assessed the correlation between fetal macrosomia and abnormal OGTT in pregnant women with term gestation and negative glucose challenge test (GCT) at 24-28 weeks. They recruited patients who had estimated fetal weights >90th percentile and a negative 50g GCT. From 170 women over a five-month period, they found that 10 patients or 5.9% had "impaired glucose metabolism at term." In this group, "we found no correlation between GCT values at 24-28 weeks, family history of diabetes mellitus, the patient's [body mass index] or weight at term, and the diagnosis of impaired glucose metabolism." Furthermore, there was no statistically significant difference in mean fetal weight between those with normal versus abnormal OGTT. This demonstrated the lack of clinical utility of using OGTT at term for predicting the incidence of fetal macrosomia. The researchers suggested utilizing a larger scale study to solidify or contradict these conclusions (Geifman-Holtzman et al., 2010).

IV. Guidelines and Recommendations

The American Diabetes Association (ADA)

The ADA publishes an extensive yearly guideline encompassing the standards of medical care in diabetes. The 2022 recommendations state:

Screening for and diagnosis of diabetes (Chapter [Ch] 2) (American Diabetes Association Professional Practice, 2021a):

- Criteria for testing for diabetes or prediabetes in asymptomatic adult:
    - Testing should be considered in overweight or obese (BMI ≥25 kg/m^2 or ≥23 kg/m^2 in Asian Americans) adults who have one or more of the following risk factors:

Diabetes Mellitus Testing, continued

- First-degree relative with diabetes
- High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
- History of CVD
- Hypertension (≥140/90 mmHg or on therapy for hypertension)
- HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
- Women with polycystic ovary syndrome
- Physical inactivity
- Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)

- Patients with prediabetes (A1c ≥5.7% [39 mmol/mol], IGT [impaired glucose tolerance], or IFG [impaired fasting glucose]) should be tested yearly.
- Women who were diagnosed with GDM should have lifelong testing at least every 3 years.
- For all other patients, testing should begin at age 45 years.
- If results are normal, testing should be repeated at a minimum of 3-year intervals, with consideration of more frequent testing depending on initial results and risk status.
- People with HIV.

- Diabetes may be diagnosed based on plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT) or A1c criteria where A1c ≥6.5% (48 mmol/mol).

<u>A1c</u>

- "To avoid misdiagnosis or missed diagnosis, the A1c test should be performed using a method that is certified by the NGSP and standardized to the Diabetes Control and Complications Trial (DCCT) assay. Grade **B**"

Diabetes Mellitus Testing, continued

- "Marked discordance between measured A1c and plasma glucose levels should raise the possibility of A1c assay interference and consideration of using an assay without interference or plasma blood glucose criteria to diagnose diabetes. Grade **B**"

- "In conditions associated with an altered relationship between A1c and glycemia, such as hemoglobinopathies, including sickle cell disease, pregnancy (second and third trimesters and the postpartum period), glucose-6-phosphate dehydrogenase deficiency, HIV, hemodialysis, recent blood loss or transfusion, or erythropoietin therapy, only plasma blood glucose criteria should be used to diagnose diabetes. Grade **B**"

- "Adequate carbohydrate intake (at least 150 g/day) should be assured for 3 days prior to oral glucose tolerance testing as a screen for diabetes. Grade **A**"

Prediabetes and Type 2 Diabetes

- "Screening for prediabetes and type 2 diabetes with an informal assessment of risk factors or validated risk calculator should be done in asymptomatic adults. Grade **B**"
- "Testing for prediabetes and/ or type 2 diabetes in asymptomatic people should be considered in adults of any age with overweight or obesity (BMI ≥25 kg/m^2 or ≥23 kg/m^2 in Asian Americans) who have one or more risk factors. Grade **B**"
- "For all people screening should begin at age 35 years. Grade **B**"
- "If tests are normal, repeat screening recommended at a minimum of 3-year intervals is reasonable, sooner with symptoms or change in risk (i.e., weight gain). Grade **C**"
- "To screen for prediabetes and type 2 diabetes, fasting plasma glucose, 2-h plasma glucose during 75-g oral glucose tolerance test, and A1C are each appropriate. Grade **B**"

- "When using oral glucose tolerance testing as a screen for diabetes, adequate carbohydrate intake (at least 150 g/ day) should be assured for 3 days prior to testing. Grade **A**"
- "In people with prediabetes and type 2 diabetes, identify and treat cardiovascular disease risk factors. Grade **A**"
- "Risk-based screening for prediabetes and/or type 2 diabetes should be considered after the onset of puberty or after 10 years of age, whichever occurs earlier, in children and adolescents with overweight (BMI ≥85th percentile) or obesity (BMI ≥95th percentile) and who have one or more risk factor for diabetes. Grade **B**"
- "People with HIV should be screened for diabetes and prediabetes with a fasting glucose test before starting antiretroviral therapy, at the time of switching antiretroviral therapy, and 3-6 months after starting or switching antiretroviral therapy. If initial screening results are normal, fasting glucose should be checked annually. Grade **E**"

Cystic Fibrosis-Related Diabetes

- "Annual screening for cystic fibrosis-related diabetes with an oral glucose tolerance test should begin by age 10 years in all patients with cystic fibrosis not previously diagnosed with cystic fibrosis-related diabetes. Grade **B**"

Diabetes Mellitus Testing, continued

- "A1c is not recommended as a screening test for cystic fibrosis–related diabetes. Grade **B**"
- "Beginning 5 years after the diagnosis of cystic fibrosis–related diabetes, annual monitoring for complications of diabetes is recommended. Grade **E"**

Gestational Diabetes Mellitus

- "In women who are planning pregnancy, screen those with risk factors (**Grade B**) and consider testing all women with undiagnosed diabetes (**Grade E**).
- "Before 15 weeks of gestation, test women with risk factors **B** and consider testing all women **E** for undiagnosed diabetes at the first prenatal visit in those with risk factors using standard diagnostic criteria, if not screened preconception."
- "Before 15 weeks of gestation, screen for abnormal glucose metabolism to identify women who are at higher risk of adverse pregnancy and neonatal outcomes, are more likely to need insulin, and are at high risk of a later gestational diabetes mellitus diagnosis. Grade **B**."
- "Screen for early abnormal glucose metabolism using fasting glucose of 110–125 mg/dL (6.1 mmol/L) or A1C 5.9–6.4% (41–47 mmol/mol). Grade **B**"
  "Screen for gestational diabetes mellitus at 24–28 weeks of gestation in pregnant women not previously found to have diabetes or high-risk abnormal glucose metabolism detected earlier in the current pregnancy. Grade **A**" (American Diabetes Association Professional Practice, 2021a).

On diagnostic tests for diabetes (Ch 2):

"The FPG and 2-h PG may be used to diagnose diabetes. The concordance between the FPG and 2-h PG tests is imperfect, as is the concordance between A1C and either glucose-based test. Compared with FPG and A1C cut points, the 2-h PG value diagnoses more people with prediabetes and diabetes. In people in whom there is discordance between A1C values and glucose values, FPG and 2-h PG are more accurate."

"The A1c test should be performed using a method that is certified by the NGSP (www.ngsp.org) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. Point-of-care A1c assays may be NGSP certified and cleared by the U.S. Food and Drug Administration (FDA) for use in monitoring glycemic control in people with diabetes in both Clinical Laboratory Improvement Amendments (CLIA)-regulated and CLIA-waived settings. proficiency testing is not always mandated for performing the test. Point-of-care A1C assays have not been prospectively studied for the diagnosis of diabetes and are not recommended for diabetes diagnosis; if used, they should be confirmed with a validated measure. In the U.S., point-of-care A1C is a laboratory test that limits CLIA regulation…point-of-care A1C assays may be more generally applied for assessment of glycemic control in the clinic" (ADA, 2021a).

Diabetes Mellitus Testing, continued

Comorbidities (Ch 4) (ADA, 2020a, 2021b)

"Individuals with HIV are at higher risk for developing prediabetes and diabetes on antiretroviral (ARV) therapies, so a screening protocol is recommended. The A1c test may underestimate glycemia in people with HIV; it is not recommended for diagnosis and may present challenges for monitoring." (ADA, 2021b)

Glycemic Targets (Ch 6) (ADA, 2020b, 2021c)

- "Assess glycemic status (A1C or other glycemic measurement such as time in range or glucose management indicator) at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control). Grade **E**"
- "Assess glycemic status at least quarterly and as needed in patients whose therapy has recently changed and/or who are not meeting glycemic goals. Grade **E**"
- "An A1c goal for many non-pregnant adults of <7% (53 mmol/mol) without significant hypoglycemia is appropriate. Grade **A**"
- "On the basis of provider judgment and patient preference, achievement of lower A1C levels than the goal of 7% may be acceptable and even beneficial if it can be achieved safely without significant hypoglycemia or other adverse effects of treatment. Grade **B**"
- "Less stringent A1C goals (such as <8% [64 mmol/mol] may be appropriate for patients with limited life expectancy or where the harms of treatment are greater than the benefits. Grade **B**" (American Diabetes Association Professional Practice, 2021b).

Children & Adolescents (Ch 13) (ADA, 2020c, 2021d)

- The traditional idea of type 2 diabetes occurring only in adults and type 1 diabetes occurring only in children is no longer accurate, as both diseases can occur in both age-groups. The recommendations concerning diabetes testing for children and adolescents are as follows:
    - "Fasting plasma glucose, 2-h plasma glucose during a 75-g oral glucose tolerance test, and A1c can be used to test for prediabetes or diabetes in children and adolescents. Grade **B"**
    - "Although A1c is not recommended for diagnosis of diabetes in children with cystic fibrosis or symptoms suggestive of acute onset of type 1 diabetes and only A1c assays without interference are appropriate for children with hemoglobinopathies, ADA continues to recommend A1c for diagnosis of type 2 diabetes in this population (ungraded)"
    - "If screening is normal, repeat testing at a minimum of 3-year intervals **E**, or more frequently if BMI is increasing. **C"**

- "A1C goals must be individualized and reassessed over time. An A1C of <7% (53 mmol/mol) is appropriate for many children. **B**"

- Concerning screening of asymptomatic children and adolescents (under the age of 18 but after the onset of puberty or after 10 years of age, whichever occurs earlier) for type 2 diabetes or prediabetes, the ADA recommends the following (ADA, 2021d; American Diabetes Association Professional Practice, 2021c):
  - Criteria: Consider testing in youth "who have [sic] overweight (≥85th percentile) or obesity (≥95th percentile) and who have one or more additional risk factors for diabetes:
    - Maternal history of diabetes or GDM during the child's gestation-Grade **A**
    - Family history of type 2 diabetes in first- or second-degree relative-Grade **A**
    - Race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)-Grade **A**
    - Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational-age birth weight)-Grade **B**

Pregnancy (Ch 14) (ADA, 2021e)

- "…although A1c may be useful, it should be used as a secondary measure of glycemic control in pregnancy, after self-monitoring of blood glucose."

- "Fasting and postprandial self-monitoring of blood glucose are recommended in both gestational diabetes mellitus and preexisting diabetes in pregnancy to achieve optimal glucose levels. Glucose targets are fasting plasma glucose <95 mg/dL (5.3 mmol/L) and either 1-h postprandial glucose <140 mg/dL (7.8 mmol/L) or 2-h postprandial glucose <120 mg/dL (6.7 mmol/L). Some women with preexisting diabetes should also test blood glucose preprandially. Grade **B**"

- "Due to increased red blood cell turnover, A1c is slightly lower in normal pregnancy than in normal nonpregnant women. Ideally, the A1c target in pregnancy is <6% (42 mmol/mol) if this can be achieved without significant hypoglycemia, but the target may be relaxed to <7% (53 mmol/mol) if necessary to prevent hypoglycemia"

- "Given the alteration in red blood cell kinetics during pregnancy and physiological changes in glycemic parameters, A1c levels may need "to be monitored more frequently than usual (e.g., monthly)."

- "The OGTT is recommended over A1C at 4–12 weeks postpartum because A1C may be persistently impacted (lowered) by the increased red blood cell turnover related to pregnancy, by blood loss at delivery, or by the preceding 3-month glucose profile. The OGTT is more sensitive at detecting glucose intolerance, including both prediabetes and diabetes."

- "Because GDM often represents previously undiagnosed prediabetes, type 2 diabetes, maturity-onset diabetes of the young, or even developing type 1 diabetes, women with GDM should be

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tested for persistent diabetes or prediabetes at 4–12 weeks postpartum with a fasting 75-g OGTT using nonpregnancy criteria as outlined in Section 2, "Classification and Diagnosis of Diabetes"."

- " In the absence of unequivocal hyperglycemia, a positive screen for diabetes requires two abnormal values. If both the fasting plasma glucose (≥126 mg/dL [7.0 mmol/L]) and 2-h plasma glucose (≥200 mg/dL [11.1 mmol/L]) are abnormal in a single screening test, then the diagnosis of diabetes is made. If only one abnormal value in the OGTT meets diabetes criteria, the test should be repeated to confirm that the abnormality persists."

- "Because GDM is associated with an increased lifetime maternal risk for diabetes estimated at 50–70% after 15–25 years, women should also be tested every 1–3 years thereafter if the 4–12 weeks postpartum 75-g OGTT is normal. Ongoing evaluation may be performed with any recommended glycemic test (e.g., annual A1C, annual fasting plasma glucose, or triennial 75-g OGTT using nonpregnant thresholds)." (American Diabetes Association Professional Practice, 2021d).

Diabetes Canada Clinical Practice Guidelines Expert Committee

This Expert Committee published a comprehensive guideline on the prevention and management of diabetes. Relevant items, recommendations, and comments—particularly those relating to the use of A1c testing—are captured below:

- "Screen for type 2 diabetes using a fasting plasma glucose and/or glycated hemoglobin (A1C) every 3 years in individuals ≥40 years of age or in individuals at high risk on a risk calculator (33% chance of developing diabetes over 10 years)."
- "In the absence of evidence for interventions to prevent or delay type 1 diabetes, routine screening for type 1 diabetes is not recommended."
- "For most individuals with diabetes, A1C should be measured approximately every 3 months to ensure that glycemic goals are being met or maintained. In some circumstances, such as when significant changes are made to therapy, or during pregnancy, it is appropriate to check A1C more frequently. Testing at least every 6 months should be performed in adults during periods of treatment and healthy behavior stability when glycemic targets have been consistently achieved."
- A1C can be misleading in various medical conditions ("e.g. hemoglobinopathies, iron deficiency, hemolytic anemia, severe hepatic or renal disease") and should not be used for " diagnostic use in children and adolescents (as the sole diagnostic test), pregnant women as part of routine screening for gestational diabetes, those with cystic fibrosis or those with suspected type 1 diabetes"
- Diabetes "should" be diagnosed at a level of A1C ≥6.5%.
- "Screening for diabetes using FPG and/or A1C should be performed every 3 years in individuals ≥40 years of age or at high risk using a risk calculator [Grade D, Consensus]. Earlier testing and/or more frequent follow up (every 6 to 12 months) with either FPG and/or A1C should be considered in those at very high risk using a risk calculator or in people with additional risk factors for diabetes [Grade D, Consensus]"

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- It should be mentioned that "Glycemic targets should be individualized [Grade D, Consensus]" based upon various considerations including, but not limited to, the patient's functional dependence, medical history, life expectancy, and life course stage. Moreover, the grading of recommendations above (e.g., "Grade D") reflect the methodological rigor used at arriving at the conclusion, such that lower grades reflect the presence of weaker evidence. But though the "paucity of clinical evidence addressing the areas of therapy, prevention, diagnosis or prognosis precluded the assignment of a higher grade", the authors recognize and note that many Grade D recommendations are "very important to the contemporary management of diabetes (Committee, 2018).

The United States Preventive Services Task Force (USPSTF)

The USPSTF recommends screening overweight or obese adults ages 40-70 years for abnormal blood glucose, with a grade B recommendation. In it, they recommend hemoglobin A1c as one of the screening tests (USPSTF, 2016).

The USPSTF has also provided guidelines pertaining to the screening of gestational diabetes. For asymptomatic pregnant persons at 24 weeks gestation or after, with a letter "B" grade, the USPSTF recommends screening for gestational diabetes in this population. However, in asymptomatic pregnant persons before 24 weeks gestation, the USPSTF states that "current evidence is insufficient to assess the balance of benefits and harms of screening" and has given it an "I" grade (USPSTF, 2021).

World Health Organization (WHO)

The Global Report on Diabetes (WHO, 2016) states that: "Glycated haemoglobin (HbA1c) is the method of choice for monitoring glycaemic control in diabetes. An advantage of using HbA1c is that the patient does not need to be in a fasting state. Ideally it should be measured twice a year in people with type 2 diabetes and more frequently in those with type 1 diabetes. However, HbA1c testing is more costly than glucose measurement, and therefore less readily available. If HbA1c testing is not available, fasting or post-meal blood glucose is an acceptable substitute."

The WHO also published a "module" titled "Hearts-D: Diagnosis and Management of Type 2 Diabetes in 2020. In it, a testing algorithm for "treatment of type 2 diabetes mellitus with insulin" is included at the bottom. The algorithm calls for an HbA1c assessment to be performed "in 3 months" if the patient is stabilized as a result of the insulin treatment. (WHO, 2020)

The National Academy of Clinical Biochemistry (NACB)

The NACB guidelines (NACB, 2011) state:

Diabetes Mellitus Testing, continued

- "Laboratories should use only Hb A1c assay methods that are certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the DCCT reference. The manufacturers of Hb A1c assays should also show traceability to the IFCC reference method."

- "Laboratories that measure HbA1c should participate in a proficiency-testing program, such as the College of American Pathologists (CAP) HbA1c survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network."

- "HbA1c testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or who are not meeting treatment goals."

- "HbA1c may be used for the diagnosis of diabetes, with values >6.5% being diagnostic. An NGSP-certified method should be performed in an accredited laboratory. Analogous to its use in the management of diabetes, factors that interfere with or adversely affect the Hb A1c assay will preclude its use in diagnosis."

- "Point-of-care HbA1c assays are not sufficiently accurate to use for the diagnosis of diabetes."

American Academy of Family Physicians (AAFP)
In 2022, the AAFP published a clinical summary of the USPSTF recommendation for screening for prediabetes and type 2 diabetes mellitus. The document deferred to the USPSTF recommendations, with the testing audience being "Nonpregnant adults aged 35 to 70 years who have overweight or obesity and no symptoms of diabetes"—a move from 40 years of age in the previous recommendation—while deeming screening every 3 years to be a reasonable approach (AAFP, 2022).

Endocrine Society

The Endocrine Society published this guideline regarding management of diabetes in older adults. In it, they recommend screening for prediabetes or diabetes every 2 years for patients 65 years or older. Fasting plasma glucose and/or HbA1c may be used. However, the Society does recommend caution when interpreting HbA1c results, as older patients are more likely to have conditions that alter red blood cell turnover. (LeRoith et al., 2019)

National Institute for Health and Care Excellence (NICE)

Measure HbA1c levels in adults with type 2 diabetes every:
- 3 to 6 months (tailored to individual needs) until HbA1c is stable on unchanging therapy
- 6 months once the HbA1c level and blood glucose lowering therapy are stable."

"Measure HbA1c using methods calibrated according to International Federation of Clinical Chemistry (IFCC) standardisation."

"If HbA1c monitoring is invalid because of disturbed erythrocyte turnover or abnormal haemoglobin type, estimate trends in blood glucose control using one of the following:
- quality-controlled plasma glucose profiles

- total glycated haemoglobin estimation (if abnormal haemoglobins)
- fructosamine estimation."

"Investigate unexplained discrepancies between HbA1c and other glucose measurements. Seek advice from a team with specialist expertise in diabetes or clinical biochemistry." (NICE, 2022)

American Association of Clinical Endocrinologists (AACE)

The AACE provides the following inclusion criteria for individuals who should be screened for prediabetes or type 2 diabetes:

- Age ≥45 years without other risk factors
- CVD or family history of T2D
- Overweight or obese
- Sedentary lifestyle
- Member of an at-risk racial or ethnic group:
  - Asian
  - African American
  - Hispanic
  - Native American (Alaska Natives and American Indians)
  - Pacific Islander
- High-density lipoprotein cholesterol (HDL-C) <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
- Impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and/or metabolic syndrome
- Polycystic ovary syndrome (PCOS), acanthosis nigricans, or nonalcoholic fatty liver disease (NAFLD)
- Hypertension (blood pressure >140/90 mm Hg or on antihypertensive therapy)
- History of gestational diabetes or delivery of a baby weighing more than 4 kg (9 lb)
- Antipsychotic therapy for schizophrenia and/or severe bipolar disease
- Chronic glucocorticoid exposure
- Sleep disorders in the presence of glucose intolerance (A1C >5.7%, IGT, or IFG on previous testing), including obstructive sleep apnea (OSA), chronic sleep deprivation, and night-shift occupation

The AACE recommends repeat testing at least every 3 years for individuals with normal results. Consider annual screening for patients with 2 or more risk factors.

In a 2022 update focusing on developing a diabetes mellitus comprehensive care plan, the AACE expounds on how the diagnosis of diabetes mellitus should be made. According to the authors, the ELs refer to evidence levels established by AACE evidence ratings, where "descriptors of "must," "should," and "may" generally but not strictly correlate with Grade A (strong), Grade B (intermediate), and Grade C (weak) recommendations, respectively" (Blonde et al., 2022). The relevant recommendations are captured below.

"Recommendation 1.1

Diabetes Mellitus Testing, continued

The diagnosis of DM is based on the following criteria…:

- FPG concentration ≥126 mg/dL (after ≥8 hours of an overnight fast), or
- Plasma glucose (PG) concentration ≥200 mg/dL 2 hours after ingesting a 75-g oral glucose load after an overnight fast of at least 8 hours, or
- Symptoms of hyperglycemia (eg, polyuria, polydipsia, polyphagia) and a random (nonfasting) PG concentration ≥200 mg/dL, or
- A1C level ≥6.5%

Diagnosis of DM requires 2 abnormal test results, either from the same sample or 2 abnormal results on samples drawn on different days. However, a glucose level ≥200 mg/dL in the presence of symptoms for DM confirms the diagnosis of DM.

Grade A; BEL 2 and expert opinion of task force

Recommendation 1.2

Prediabetes is identified by the presence of impaired fasting glucose (IFG) (100 to 125 mg/dL), impaired glucose tolerance (IGT), which is a PG value of 140 to 199 mg/dL 2 hours after ingesting 75 g of glucose, and/or A1C value between 5.7% and 6.4% (Table 4). A1C should be used only for screening for prediabetes. The diagnosis of prediabetes, which may manifest as either IFG or IGT, should be confirmed with glucose testing.

Grade B; BEL 2

Recommendation 1.3

T1D is characterized by marked insulin deficiency in the presence of hyperglycemia and positive autoantibody tests to glutamic acid decarboxylase (GAD65), pancreatic islet β cells (tyrosine phosphatase IA-2), and IA-2b zinc transporter (ZnT8), and/or insulin. The presence of immune markers and clinical presentation are needed to establish the correct diagnosis and to distinguish between T1D and T2D in children or adults, as well as to determine appropriate treatment.

Grade A; BEL 2

Recommendation 1.4

T2D is characterized by progressive loss of β-cell insulin secretion and variable defects in insulin sensitivity. T2D is often asymptomatic and can remain undiagnosed for many years; therefore, all adults ≥35 years of age with risk factors should be screened for DM (Table 5).

Grade A; BEL 1

Recommendation 1.5

Diabetes Mellitus Testing, continued

GDM is defined as carbohydrate intolerance that begins or is first recognized during pregnancy and resolves postpartum. Pregnant women with risk factors for DM should be screened at the first prenatal visit for undiagnosed T2D using standard criteria (Table 4).

Grade B; BEL 1

Recommendation 1.6

Screen all pregnant women for GDM at 24 to 28 weeks' gestation. Diagnose GDM with either the one-step or the two-step approach.

- The one-step approach uses a 2-hour 75-g oral glucose tolerance test (OGTT) after ≥8 hours of fasting with diagnostic cutoffs of one or more FPG ≥92 mg/dL, 1-hour PG ≥180 mg/dL, or 2-hour PG ≥153 mg/dL.
- The two-step approach uses a nonfasting 1-hour 50-g glucose challenge test with 1-hour PG screening threshold of 130 or 140 mg/dL. For women with a positive screening test, the 3-hour 100-g OGTT is used for diagnosis with 2 or more PG tests that meet the following thresholds: FPG ≥95 mg/dL, 1-hour ≥180 mg/dL, 2-hour ≥155 mg/dL, 3-hour ≥140 mg/dL.

Grade A; BEL 1

Recommendation 1.7

Clinicians should consider evaluation for monogenic DM in any child or young adult with an atypical presentation, clinical course, or response to therapy. Monogenic DM includes neonatal diabetes and nonautoimmune diabetes of multiple genetic causes, also known as maturity-onset diabetes of the young (MODY). Most children with DM occurring under age 6 months of age have a monogenic cause as autoimmune T1D rarely occurs before 6 months of age. Other monogenic forms of diabetes are characterized by mutation of genes of transcription factors, genes regulating pancreatic development or atrophy, abnormal insulin genes, genes related to endoplasmic reticulum stress that impair insulin secretion, or abnormal glucokinase genes that cause impaired insulin signaling.

Grade B; BEL 2

Although not expressly listed as recommendations for diabetes screening, some additional information of note is that

"A glucose level ≥200 mg/dL in the presence of hyperglycemia symptoms such as polyuria and polydipsia confirm the diagnosis of DM. In individuals with discordant results from 2 different tests, the test result that is above the diagnostic cut point should be repeated on a different day."

"In view of physiological changes in pregnancy that could affect glycated hemoglobin levels, A1C should not be used for GDM screening or diagnosis of DM."

Diabetes Mellitus Testing, continued

"All pregnant women should be screened for GDM at 24 to 28 weeks' gestation. Universal screening is recommended, as selective screening (only in women with risk factors) would miss a significant number of women with GDM and universal screening has been shown to be cost-effective compared with selective screening" (Blonde et al., 2022).

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

The 2019 Consensus statement from the AACE/ACE on the Management of Type 2 Diabetes states:

- The hemoglobin A1c (A1c) target should be individualized based on numerous factors such as age, life expectancy, comorbid conditions, duration of diabetes, risk of hypoglycemia or adverse consequences from hypoglycemia, patient motivation, and adherence.

- An A1c level of ≤6.5% is considered optimal if it can be achieved in a safe and affordable manner, but higher targets may be appropriate for certain individuals and may change for a given individual over time.

- Therapy must be evaluated frequently (e.g., every 3 months) until stable using multiple criteria, including A1c, SMBG records (fasting and postprandial) or continuous glucose monitoring tracings, documented and suspected hypoglycemia events, lipid and BP values, adverse events (weight gain, fluid retention, hepatic or renal impairment, or CVD), comorbidities, other relevant laboratory data, concomitant drug administration, complications of diabetes, and psychosocial factors affecting patient care. Less frequent monitoring is acceptable once targets are achieved (Garber et al., 2020)

Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Working Group

KDIGO published recommendations on diabetes and chronic kidney disease (CKD). They recommend using HbA1c to monitor diabetic and CKD patients twice a year or as often as 4 times a year if glycemic target is not met or a change is made in therapy. KDIGO advises that "accuracy and precision of HbA1c measurement declines with advanced CKD, particularly among patients treated by dialysis, in whom HbA1c measurements have low reliability." They also recommend an "individualized HbA1c target ranging from <6.5% to <8.0% in patients with diabetes and CKD not treated with dialysis" (Rossing et al., 2022).

V. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

A search for "Hemoglobin A1c" on the FDA website yielded 42 results on April 28, 2022. (FDA, 2022). 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

Diabetes Mellitus Testing, continued

Code Number	Code Description
82951	Glucose; tolerance test (GTT), 3 specimens (includes glucose)
82952	Glucose; tolerance test, each additional beyond 3 specimens
82985	Glycated protein
83036	Hemoglobin; glycosylated (A1C)
83037	Hemoglobin; glycosylated (A1C) by device cleared by FDA for home use

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
8/19/22	Added coverage criteria #6a and modified wording in overall coverage criteria to align with updated clinical standards.
10/16/23	Renamed policy as "Diabetes Mellitus Testing" (was previously titled as "Hemoglobin A1c"), and expanded coverage criteria to address testing beyond Hemoglobin A1c indications alone.

Diabetes Mellitus Testing, continued

Hepatitis Testing

Policy #: AHS – G2036	**Prior Policy Name & Number (as applicable):** Hepatitis C (AHS-G2036)
Implementation Date: 9/15/21	**Date of Last Revision:** 8/19/22, 10/16/23, 1/8/24 (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

Infectious hepatitis is an inflammation of the liver caused by the hepatitis viruses. Hepatitis C is a blood-borne virus that can be spread via sharing needles or other equipment to inject drugs as well as in inadequate infection control in healthcare settings (CDC, 2018).

Hepatitis C causes liver disease and inflammation. A chronic HCV infection can lead to hepatic damage, including cirrhosis and hepatocellular carcinoma, and is the most common cause of liver transplantation in the United States (AASLD-IDSA, 2015).

Hepatitis B is spread by the "Percutaneous, mucosal, or nonintact skin exposure to infectious blood, semen, and other body fluids." As the hepatitis B virus is concentrated most highly in blood, "percutaneous exposure is an efficient mode of transmission", though HBV can also be transmitted through birth to an infected mother and sexual contact with an infected person and less commonly through needle-sticks or other sharp instrument injuries, organ transplantation and dialysis, and interpersonal contact through sharing items, such as razors or toothbrushes or contact with open sores of an infected person. Similar to HCV infection, 15% to 25% of people with chronic HBV infection develop chronic liver disease (CDC, 2020a).

The general route of transmission for the hepatitis A virus is through the fecal-oral route by close person-to-person contact with an infected person, sexual contact with an infected person, or the ingestion of contaminated food or water, with the bloodborne transmission of HAV being uncommon (CDC, 2020a). Though death is uncommon and most people with acute HAV infection recover with no lasting liver damage, HAV remains a worldwide public health issue and is endemic in many low- to middle-income countries (CDC, 2020a; Keles et al., 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.

Hepatitis B

1. For all individuals 18 years of age and older, triple panel testing (hepatitis B surface antigen [HBsAg], hepatitis B surface antibody [anti-HBs], total antibody to hepatitis B core antigen [anti-HBc]) for Hepatitis B (HBV) infection once per lifetime **MEETS COVERAGE CRITERIA**.

2. For asymptomatic, non-pregnant individuals, the following annual HBV infection screening **MEETS COVERAGE CRITERIA**:

a) HBsAg and hepatitis B surface antibody (anti-HBs) for infants born from an HBsAg-positive individual.

b) Triple panel testing (HBsAg, anti-HBs, anti-HBc) when one of the following high-risk situations is met:

i) For individuals born in or who have recently traveled to geographic regions with a HBV prevalence 2% or higher (see Note 1).

ii) For U.S.-born individuals not vaccinated as infants whose parents were born in geographic regions with a HBV prevalence 8% or higher (see Note 1).

iii) For individuals with a history of incarceration.

iv) For individuals affected with HIV

v) For individuals infected with HIV

vi) For men who have sex with men

vii) For household contacts, needle-sharing contacts, and sex partners of HBV-infected individuals.

viii) For injection-drug users

ix) For individuals with an active hepatitis C virus infection or who have a history of hepatitis C virus infection

x) For individuals with elevated liver enzymes

Hepatitis Testing, continued

xi) For individuals who are on long-term hemodialysis treatment

xii) For individuals with diabetes

xiii) For healthcare and public safety workers exposed to blood or body fluids

3. For individuals who test positive for anti-HBc, follow up IgM antibody to anti-HBc (IgM anti-HBc) testing to distinguish between an acute or chronic infection **MEETS COVERAGE CRITERIA.**

4. For the confirmation of seroconversion after hepatitis B vaccination, anti-HBs testing **MEETS COVERAGE CRITERIA.**

5. For individuals who test positive for HBV by initial antibody screening and who will undergo immunosuppressive drug therapy, HBV DNA testing **MEETS COVERAGE CRITERIA**.

Hepatitis C

6. For all individuals 18 years of age and older, antibody testing for Hepatitis C (HCV) infection once per lifetime **MEETS COVERAGE CRITERIA.**

7. For any individual with the following recognized conditions or exposures, one-time, post-exposure antibody testing for HCV infection **MEETS COVERAGE CRITERIA**:

a) For individuals who have used illicit intranasal or injectable drugs.

b) For individuals who have received clotting factor concentrates produced before 1987.

c) For individuals with a history of hemodialysis.

d) For individuals with evidence of liver disease (based on clinical presentation, persistently abnormal ALT levels, or abnormal liver function studies).

e) For individuals infected with HIV.

f) For individuals who received an organ transplant before July 1992.

g) For individuals who received a blood transfusion or blood component before July 1992.

h) For individuals notified that they received blood from a donor who later tested positive for an HCV infection.

i) For individuals with a history of incarceration.

j) For individuals who received a tattoo in an unregulated setting.

k) For healthcare, emergency medical, and public safety workers after needle sticks, sharps, or mucosal exposures to HCV-positive blood.

l) For children born from an HCV-positive individual.

m) For current sexual partners of HCV-infected persons.

8. Routine periodic antibody testing for HCV **MEETS COVERAGE CRITERIA** for individuals with any of the following ongoing risk factors (while risk factors persist):

   a) For individuals who currently inject drugs and share needles, syringes, or other drug preparation equipment.

   b) For individuals who are receiving ongoing hemodialysis.

   c) For individuals engaging in high-risk sexual behavior.

9. For individuals who test positive for HCV by initial antibody screening, follow up nucleic acid testing for HCV (to differentiate between active infection and resolved infection) **MEETS COVERAGE CRITERIA.**

10. Prior to the initiation of direct acting anti-viral (DAA) treatment, one time testing for HCV genotype to guide selection of the most appropriate antiviral regimen **MEETS COVERAGE CRITERIA**.

11. Testing for HCV viral load with a quantitative nucleic acid test **MEETS COVERAGE CRITERIA** in **any** of the following situations:

    a) Prior to the initiation of DAA therapy.

    b) After four weeks of DAA therapy.

    c) At the end of treatment.

    d) Twelve, twenty-four, and forty-eight weeks after completion of treatment.

NOTES:

Note 1: The CDC defines HBsAg prevalence by geographic region: https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/hepatitis-b.

III. Scientific Background

Hepatitis C

The Centers for Disease Control and Prevention estimate that 2.4 million people in the United States have chronic hepatitis C (CDC, 2020b). Prevalence of the infection is highest in individuals born between 1945 and 1965. This rate is approximately six times higher than that seen in other adult age groups, and the CDC estimated approximately 50,300 new infections occurring each year (CDC, 2018). Hepatitis C virus (HCV) infection is the most common reason for liver transplantation in adults in the U.S. and may lead to hepatocellular carcinoma (Chopra, 2023).

It is estimated that 20% of people with HCV infection will develop cirrhosis, and nearly 5% will die from liver disease resulting from the HCV infection. The number of deaths from hepatitis is increasing and is projected to continue to increase for several more decades unless treatment is scaled up considerably (Razavi et al., 2014). Although HCV infection is common, it is estimated that 50-75% of individuals who

Hepatitis C, continued

are infected are unaware of their infection as symptoms are absent or nonspecific until much later, and therefore do not receive the care and treatment that can mitigate progression to severe liver disease and possibly death (Hagan et al., 2006; Rein et al., 2012).

Hepatitis C virus is spread through exposure to blood of infected individuals. Such exposure includes injection drug use, blood transfusions (prior to 1992), and to a lesser extent, high-risk sexual behaviors. Additionally, being born to an HCV-infected mother, hemodialysis, intranasal drug use, tattoos, incarceration, needle sticks, and invasive procedures (prior to implementation of universal precautions) are also associated with increased risk of HCV infection. Some countries are experiencing a recent resurgence of HCV infection among young intravenous drug users and HIV-infected homosexual men (CDC, 2015a; Wandeler et al., 2015).

Hepatitis C virus is a small, positive-stranded RNA-enveloped virus with a highly variable genome (Simmonds, 2001). Assessment of the HCV genotype is crucial for management of the HCV infection. There are currently six major genotypes of HCV, and major treatment decisions (regimen, dosing, duration) vary from genotype to genotype (Chopra & Arora, 2022a). Some regimens for one genotype (such as ledipasvir-sofosbuvir [“Harvoni”] for genotype 1) may not be effective for another (in this case, Harvoni may be used for genotypes 1, 4, 5, and 6 but not 2 or 3) (Andrew J Muir, 2023; Lexicomp, 2022).

Hepatitis C virus is frequently asymptomatic, necessitating the need of strong screening procedures. As many as 50% of HCV-infected individuals are unaware of their diagnosis, and risk factors such as drug use or blood transfusions may increase risk of acquiring an HCV infection. Several expert groups, such as the CDC, have delineated screening recommendations in order to provide better care against the virus (S. Chopra, Arora, Sanjeev, 2018).

Hepatitis C can be diagnosed with either serologic antibody assays or molecular RNA tests. A serologic assay can detect an active infection and a resolved HCV infection, but cannot differentiate whether the infection is acute, chronic, or no longer present. Various serologic assays include enzyme immunoassays (EIA), chemiluminescence immunoassays (CIA), and point-of-care rapid immunoassays (Spach, 2020).

Molecular RNA tests detect Hepatitis C RNA, and the process includes nucleic acid test (NAT) or nucleic acid amplification test (NAAT). The NAT test becomes positive 1 to 2 weeks after initial infection and it has become the gold standard test for patients who have a positive EIA screening test. The NAT can detect whether a patient has a current active infection or a resolved infection (Spach, 2020).

Hepatitis B

The hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the hepadnavirus family. The diagnosis of its acute infection is characterized by the detection of hepatitis B surface antigen (HBsAg) and immunoglobulin M (IgM) antibody to hepatitis B core antigen (anti-HBc), and chronic conditions develop in 90% of infants after acute infection at birth, 25%–50% of children newly infected at ages 1 to 5 years, and 5% of people newly infected as adults (CDC, 2020a; Lok, 2022).

Hepatitis B virus is transmitted from infected patients to those who are not immune (i.e., hepatitis B surface antibody [anti-HBs]-negative). Methods of transmission include mother-to-child (whether in utero, at birth, or after birth), breastfeeding, paternal transmission (i.e., close contact with infected blood or fluid of fathers), transfusion, sexual transmission, nosocomial infection, percutaneous inoculation, transplantation, and blood exposure via minor breaks in skin or mucous membranes (Teo & Lok, 2022).

Hepatitis Testing, continued

In the United States, an estimated 862,000 people were living with chronic hepatitis B infection in 2016, with 21,600 new infections in 2018. Though most people with acute disease recover with no lasting liver damage, 15% to 25% of those with chronic disease develop chronic liver disease, including cirrhosis, liver failure, or liver cancer. It is believed that there are more than 250 million HBV carriers in the world, 600,000 of whom die annually from HBV-related liver diseases. As many as 60% of HBV-infected persons are unaware of their infection, and many remain asymptomatic until the presentation of cirrhosis or late-stage liver disease (CDC, 2020a; Lok, 2022; US Preventive Services Task Force, 2020).

The initial evaluation of chronic HBV infection should include a history and physical examination focusing on "risk factors for coinfection with hepatitis C virus (HCV), hepatitis delta virus (HDV), and/or HIV; use of alcohol; family history of HBV infection and hepatocellular carcinoma (HCC); and signs and symptoms of cirrhosis." Furthermore, it should employ laboratory tests, such as "a complete blood count with platelets, liver chemistry tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, alkaline phosphatase, albumin), international normalized ratio (INR), and tests for HBV replication (HBeAg, antibody to HBeAg [anti-HBe], HBV DNA", and testing for hepatitis A virus (HAV) immunity with HAV immunoglobulin G (IgG) antibody in those who are not immune. Other considerations include evaluation for other causes of liver disease, screening for HIV infection, screening for hepatocellular carcinoma (HCC), screening for fibrosis, and, in rare cases, a liver biopsy (Lok, 2022).

Hepatitis A

Hepatitis A infection is caused by the hepatitis A virus, of which humans are the only known reservoir. The HAV virus is member of the genus *Hepatovirus* in the family Picornaviridae, and other previously used names for HAV infection include epidemic jaundice, acute catarrhal jaundice, and campaign jaundice (Lai & Chopra, 2022).

The hepatitis A virus is generally transmitted through the fecal-oral route, either via person-to-person contact (e.g., transmission within households, within residential institutions, within daycare centers, among military personnel, or sexually) or consumption of contaminated food or water (consumption of undercooked foods or foods infected by food handlers). Additional modes of transmission include blood transfusion and illicit drug use, and it should be noted that maternal-fetal transmission has not yet been described (Lai & Chopra, 2022).

Globally, approximately 1.4 million new cases of HAV infection occur each year—in the United States alone, an estimated 24,900 new infections were detected in 2018. Acute infection by HAV is usually a self-limited disease, with fulminant manifestations of hepatic failure occurring in fewer than 1 percent of cases. However, symptomatic illness due to HAV still presents itself in seventy percent of adults. Consequently, "diagnosis of acute HAV infection should be suspected in patients with abrupt onset of prodromal symptoms (nausea, anorexia, fever, malaise, or abdominal pain) and jaundice or elevated serum aminotransferase levels, particularly in the setting of known risk factors for hepatitis A transmission" through detection of serum IgM anti-HAV antibodies due to its persistence throughout the duration of the disease (CDC, 2020a; Lai & Chopra, 2022).

Proprietary Testing

Hepatitis Testing, continued

Many point-of-care tests have been developed to diagnose hepatitis C efficiently. These point-of-care tests are particularly important for diagnoses in economically impoverished areas. Examples of these tests include OraQuick, TriDot and SDBioline. The OraQuick HCV test is a FDA approved point-of-care test which utilizes a fingerstick and a small whole blood sample to detect the virus. This test is reportedly more than 98% accurate and provides results in 20 minutes (OraSure, 2013). The fourth Generation HCV Tri-Dot is a rapid test which can detect all subtypes of HCV with 100% sensitivity and 98.9% specificity (JMitra&Co, 2015). This test uses human serum or plasma and can provide results in three minutes. Finally, the SDBioline HCV is an immunochromatographic rapid test that can identify HCV antibodies in human serum, plasma, or whole blood (Inc., 2023). This test uses a safe fingerstick procedure to obtain a sample.

Hepatitis panel tests have also been developed. For example, the VIDAS® Hepatitis panel by BioMérieux tests for hepatitis A, B and C in less than two hours (BioMérieux, 2022). This panel includes 11 automated assays and is a rapid, reliable and simple testing method. Legacy Health's Hepatitis Chronic Panel detects Hepatitis B and C within 24-48 hours through a CIA method (Legacy Health, 2021).

A hepatitis C vaccine is currently not available although many vaccines are under development; barriers to the development of such a vaccine include virus diversity, a lack of knowledge of the immune responses when an infection occurs, and limited models for the testing of new vaccines (Ansaldi, et al., 2014; Bailey et al., 2019). The World Health Organization hopes for a 90% reduction in new hepatitis C cases by the year 2030 (Bailey et al., 2019).

Management of HCV infection typically involves monitoring the effect of treatment. The goal of treatment is to achieve a "sustained virologic response" (SVR), which is defined as "an undetectable RNA level 12 weeks following the completion of therapy" (Chopra & Pockros 2022). This measure is a proxy for elimination of HCV RNA. The assessment schedule may vary regimen to regimen, but the viral load is generally evaluated every few weeks (Chopra & Pockros, 2022).

In 2023, the Biden-Harris administration called on Congress to embrace its proposed five-year program to eliminate hepatitis C in the United States. This five-year program was developed through extensive consultations with key stakeholders from both within and outside of the government, including patient groups, physician groups, and federal agencies. The program aims to significantly expand screening, testing, treatment, prevention, and monitoring of hepatitis C infections in the United States and specifically focuses on populations that are at the greatest risk for infection. One main priority in this national program is to accelerate the availability of point-of-care (POC) diagnostic tests. Hepatitis C RNA diagnostic POC tests are currently available outside of the United States, allowing for a test-and-treat approach in a single visit. "The administration proposal will support the Independent Technology Assessment Program, a collaboration between the Food and Drug Administration and the National Institutes of Health, the speed up clearance or approvals for such tests, just as was done by this same group for COID-19 POC tests." It is believed that the availability of such POC tests will be game-changing for hepatitis C single-visit programs, particularly in "high-impact settings such as community health centers, substance use disorder treatment clinics, correctional facilities, emergency departments, and mobile vans" (Fleurence & Collins, 2023).

Clinical Utility and Validity

Hepatitis Testing, continued

In order to determine the link between hepatitis A infection and its rare complication of acute liver failure in children in Somalia, a retrospective study was conducted on children aged 0 to 18 who were admitted to the pediatric outpatient clinic and pediatric emergency departments of the Somalia Mogadishu-Turkey Training and Research Hospital, Somali, from June 2019 and December 2019, and who were tested for HAV and had complete study data available (Keles et al., 2021). The authors found that of the 219 hepatitis A cases analyzed, 25 (11%) were diagnosed with pediatric acute liver failure (PALF) while the remaining 194 were not. It was found that children with PALF had "significantly had more prolonged PT and aPPT, and higher INR values in coagulation assays; and had higher levels of albumin in biochemical tests than the group without liver failure (for all, $p \leq 0.05$)", though no other significant differences were found based on the other laboratory parameters tested. Moreover, "Hepatic encephalopathy was observed in individuals with hepatitis A disease (12/219; 15.4%), in which PALF positive group (5/25;40%) was significantly higher compared to the non-PALF group (7/194; 4%) ($p = < 0{,}001$). The length of stay in the hospital or intensive care unit was significantly higher in children with acute liver failure ($p = 0.001$) ." As such, Keles et al. (2021) astutely notes that though "death rates of Hepatitis A infection seem to be low", HAV infection may potentially "require long-term hospitalization of patients due to the complication of acute liver failure, which causes loss of workforce, constitutes a socio-economic burden on individuals and healthcare systems, and leads to mortality in settings where referral pediatric liver transplantation centers are not available ."

Spenatto et al. (2013) screened 6194 asymptomatic patients who were requesting an STI screening for hepatitis B infection. The authors found that only "male gender, lack of employment, and birth, in medium or high endemic country, were independently associated with HBsAg positivity in multivariate analysis", and neither sexual behavior nor vaccination status are needed to target high-risk populations (Spenatto et al., 2013).

Su et al. (2022) evaluated the cost-effectiveness of implementing universal HBV screening in China to identify optimal screening strategies. By using a Markov cohort model, the researchers "simulated universal screening scenarios in 15 adult age groups between 18 and 70 years, with different years of screening implementation (2021, 2026, and 2031) and compared to the status quo (ie, no universal screening)", investigating a total of 180 different scenarios. Their work found suggested that "with a willingness-to-pay level of three times the Chinese gross domestic product (GDP) per capita (US$30 828), all universal screening scenarios in 2021 were cost-effective compared with the status quo", with the "serum HBsAg/HBsAb/HBeAg/HBeAb/HBcAb (five-test) screening strategy in people aged 18-70 years was the most cost-effective strategy in 2021" and "the two-test strategy for people aged 18-70 years became more cost-effective at lower willingness-to-pay levels ." Most importantly, they claimed that the "five-test strategy could prevent 3·46 million liver-related deaths in China over the lifetime of the cohort" and that delaying strategic intervention will reduce overall cost-effectiveness (Su et al., 2022).

Messina et al. (2015) performed a meta-analysis on the prevalence of HCV genotypes worldwide. The authors evaluated 1217 studies encompassing approximately 90% of the global population. They calculated genotype 1 to comprise 83.4 million cases (46.2% of all HCV cases), genotype 3 to comprise 54.3 million cases (30.1%), and genotypes 2, 4, and 6 to comprise a combined 22.8% cases. Genotype 5 comprised less than 1% of HCV cases. The diversity of genotypes also varied; the highest diversity is

observed in China and South-East Asia, while in some countries, such as Egypt and Mongolia, almost all HCV infections are caused by a single genotype (Messina et al., 2015).

Inoue et al. (2017) described four HCV patients whose treatment failed. These four HCV patients had received a treatment regimen of daclatasvir plus asunaprevir, which is used for genotype 1b. However, these four patients were re-tested and found to have a different genotype; 3 patients had genotype 2 and the 4th patient had genotype 1a. The authors suggested that the daclatasvir plus asunaprevir regimen was ineffective for patients without genotype 1b (Inoue et al., 2017).

Moreno et al. (2016) performed a cost analysis of expanded HCV coverage. Two scenarios were simulated, one with expanded fibrosis coverage to stage 2 fibrosis, and the other to all fibrosis cases. Over a 20-year simulation, treatment costs increased, but private payers experienced overall savings of $10 billion to $14 billion after treatment costs. A positive "spillover" benefit of $400 million to Medicare was seen in the 5-year model, and a benefit of $7 billion to Medicare was seen in the 20-year model (Moreno et al., 2016).

Linthicum et al. (2016) assessed the cost-effectiveness of expanding screening and treatment coverage over a 20-year horizon. The authors investigated three scenarios, each of which expanded coverage to a different stage of fibrosis. "Net social value" was the primary outcome evaluated, and it was calculated by the "value of benefits from improved quality-adjusted survival and reduced transmission minus screening, treatment, and medical costs." Overall, the scenario with only fibrosis stage 3 and fibrosis stage 4 covered generated $0.68 billion in social value, but the scenario with all fibrosis patients (stages 0-4) treated produced $824 billion in social value. The authors also noted that the scenario with all fibrosis stages covered created net social value by year 9 whereas the scenario with only stages 3 and 4 covered needed all 20 years to break even (Linthicum et al., 2016).

Chen et al. (2019) completed a meta-analysis to research the relationship between type 2 diabetes mellitus development and patients with a HCV infection. Studies were included from 2010 to 2019. Five types of HCV individuals were incorporated in this study including those who were "non-HCV controls, HCV-cleared patients, chronic HCV patients without cirrhosis, patients with HCV cirrhosis and patients with decompensated HCV cirrhosis" (Chen et al., 2019). HCV infection was found to be a significant risk factor for type 2 diabetes mellitus development. Further, "HCV clearance spontaneously or through clinical treatment may immediately reduce the risk of the onset and development of T2DM [type 2 diabetes mellitus] (Chen et al., 2019)."

Saeed et al. (2020) completed a systematic review and meta-analysis of health utilities for patients diagnosed with a chronic hepatitis C infection. Health utility can be defined as a measure of health-related quality or general health status. A total of 51 studies comprised of 15,053 patients were included in this study. The researchers have found that "Patients receiving interferon-based treatment had lower utilities than those on interferon-free treatment (0.647 vs 0.733). Patients who achieved sustained virologic response (0.786) had higher utilities than those with mild to moderate CHC [chronic hepatitis C]. Utilities were substantially higher for patients in experimental studies compared to observational studies (Saeed et al., 2020)." Overall, these results show that chronic hepatitis C infections are significantly harming global health status based on the measurements provided by health utility instruments.

Vetter et al. (2022) conducted a retrospective study to assess the performance of rapid diagnostic tests (RDTs) for Hepatitis C virus (HCV) infection. Thirteen RDTs were studied including the Standard Q HCV Ab

by SD Biosensor, HCV Hepatitis Virus Antibody Test by Antron Laboratories, HCV-Ab Rapid Test by Beijing Wantal Biological Pharmacy Enterprise, Rapid Anti-HCV Test by InTec, First Response HCV Card Test by Premier Medical Corporation, Signal HCV Version 3.0 by Arkray Healthcare, TRI DOT HCV by J. Mitra & Co, Modified HCV-only Ab Test by Biosynex SA, SD Bioline HCV by Abbott Diagnostics, OraQuick HCV by OraSure, Prototype HCV Ab Test by BioLytical Laboratories, Prototype DPP HCV by Chembio Diagnostic Systems, and Prototype Care Start HCV by Access Bio. 1,710 samples were evaluated in which 648 samples were HCV positive and 264 samples were also HIV positive. In the samples from HIV negative patients, most RDTs showed high sensitivity of > 98% and specificity of >99%. In HIV positive patients, sensitivity was lower with only 1 RDT reaching >95%. However, specificity was higher, with only 4 RDTs showing a specificity of <97%. The authors concluded that these tests are compliant with the World Health Organization (WHO) guidance which recommends an HCV RDT to have a sensitivity of >98% and specificity >97%. However, in HIV positive patients, the specificity remained high, but none of the tests met the WHO sensitivity criteria. The authors conclude that "these findings serve as a valuable baseline to investigate RDT performance in prospectively collected whole blood samples in the intended use settings (Vetter et al., 2022)."

In a prospective study, Chevaliez et al. (2020) evaluated the use of molecular point of care (POC) testing and dried blood spot (DBS) for HCV screening in people who inject drugs (PWID). 89 HCV-seropositive PWID were further assessed with a liver assessment, blood tests, POC HCV RNA testing, and fingerstick DBS sampling. 77 patients had paired fingerstick capillary whole blood for POC HCV RNA testing and fingerstick sampling with interpretable results, while the other 12 samples had no valid result due to low sample volume. The POC HCV RNA test detected 30 HCV-seropositive PWID and DBS sampling detected 27 HCV-seropositive PWID. The rate of invalid results using the POC test was below 10%, so it may be performed by staff without extensive clinical training in decentralizing testing location. This study also showed high concordance for detection of active HCV infection from DBS compared to the POC test. The authors conclude that the use of POC diagnostic testing and DBS sampling should be recommended as a one-step screening strategy to increase diagnosis, increase treatment, and reduce the number of visits.

In an Australian observational study, Catlett et al. (2021) evaluated the Aptima HCV Quant Dx Assay to see how well it could detect HCV RNA from fingerstick capillary dried blood spot (DBS) and venipuncture-collected samples. DBS collection would benefit marginalized populations in areas that may not have access to phlebotomy services or who may have difficult venous access. DBS has also been shown to “enhance HCV testing and linkage to care,” be easy for transport and storage, and can be used for other purposes like HCV sequencing and testing for HIV or hepatitis B simultaneously, which is useful in more resource-limited settings. From 164 participants, they found HCV RNA in 45 patients. The Aptima assay rendered a sensitivity and specificity of 100% from plasma, and a sensitivity of 95.6% and specificity of 94.1% from DBS. This demonstrated the comparable diagnostic performance of this assay when it comes to detecting active HCV infection from DBS samples and plasma samples, and hopefully the eventual use of other similar assays with similar performances.

IV. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

Hepatitis C

The CDC recommends universal hepatitis C screening for

Hepatitis Testing, continued

- "Hepatitis C screening at least once in a lifetime for all adults aged 18 years and older, except in settings where the prevalence of HCV infection (HCV RNA-positivity) is less than 0.1%"
- "Hepatitis C screening for all pregnant women during each pregnancy, except in settings where the prevalence of HCV infection (HCV RNA-positivity) is less than 0.1%"

Moreover, one-time hepatitis C testing regardless of age or setting prevalence among people with recognized conditions or exposures is recommended for the following groups:

  - People with HIV
  - People who ever injected drugs and shared needles, syringes, or other drug preparation equipment, including those who injected once or a few times many years ago
  - People with selected medical conditions, including:
    - people who ever received maintenance hemodialysis
    - people with persistently abnormal ALT levels
  - Prior recipients of transfusions or organ transplants, including:
    - people who received clotting factor concentrates produced before 1987
    - people who received a transfusion of blood or blood components before July 1992
    - people who received an organ transplant before July 1992
    - people who were notified that they received blood from a donor who later tested positive for HCV infection
  - Health care, emergency medical, and public safety personnel after needle sticks, sharps, or mucosal exposure to HCV-positive blood
  - Children born to mothers with HCV infection
- Routine periodic testing is recommended for people with ongoing risk factors, while risk factors persist:
  - People who currently inject drugs and share needles, syringes, or other drug preparation equipment
  - People with selected medical conditions, including:
    - people who ever received maintenance hemodialysis
- CDC also recommends that "Any person who requests hepatitis C testing should receive it, regardless of disclosure of risk, because many persons may be reluctant to disclose stigmatizing risks (CDC, 2020e)."

The CDC also notes that the initial HCV test should be "with an FDA-approved test for antibody to HCV." A positive result for the HCV antibody indicates either a current infection or previous infection that has resolved. For those individuals, the CDC recommends testing by an FDA-approved HCV nucleic acid test (NAT) to differentiate between active infection and resolved infection. For the identification of chronic hepatitis C virus infection among persons born between 1945 and 1965, the CDC states that. "Persons who test anti-HCV positive or have indeterminate antibody test results who are also positive by HCV NAT should be considered to have active HCV infection; these persons need referral for further medical

evaluation and care." Finally, the CDC also recommends repeat testing for individuals with ongoing risk behaviors (CDC, 2012).

The CDC published guidance for healthcare personnel with potential exposure to HCV. CDC recommends testing the source patient and the healthcare personnel. When testing the source patient, baseline testing should be performed within 48 hours after exposure by testing for HCV RNA or HCV antibodies. All HCV RNA testing should be performed with a nucleic acid test. If the source patient was HCV RNA positive or if source patient testing was not performed, baseline testing for healthcare personnel should follow the same steps through nucleic acid testing 3 to 6 weeks post-exposure. A final HCV antibody test should be performed at 4 to 6 months post-exposure to ensure a negative HCV RNA test result (CDC, 2020d).

No serologic marker for acute infection is available, but for chronic infections, CDC propounds the use of "Assay for anti-HCV" and "Qualitative and quantitative nucleic acid tests (NAT) to detect and quantify presence of virus (HCV RNA)" (CDC, 2020a).

Hepatitis B

The following holds a summary of 2023 HBV screening and testing recommendations (CDC, 2023d):

- "Screen all adults aged 18 years and older at least once in their lifetime using a triple panel test
- Screen pregnant people for hepatitis B surface antigen (HBsAg) during each pregnancy regardless of vaccination status and history of testing
- Expand periodic risk-based testing to include people incarcerated, people with a history of sexually transmitted infections or multiple sex partners, and people with hepatitis C virus infection
- Test anyone who requests HBV testing regardless of disclosure of risk"

The CDC recommends testing infants born to HBsAg positive people for HBsAg and anti-HBs seromarkers. Also, the CDC recommends HBV screening for hepatitis B surface antigen (HBsAg) for all pregnant people during each pregnancy, preferably in the first trimester, regardless of vaccination status or history of testing. Pregnant people with a history of appropriately timed triple panel screening without subsequent risk for exposure to HBV (i.e., no new HBV exposures since triple panel screening) only need HBsAg screening (CDC, 2023d).

The CDC recommends testing susceptible people periodically, regardless of age with ongoing risk for exposures, while risk for exposures persists. Including (CDC, 2023d):

- "People with a history of sexually transmitted infections or multiple sex partners
- People with hepatitis C infection or a history of hepatitis C virus infection
- People incarcerated or formerly incarcerated in a jail, prison, or other detention setting
- Infants born to HBsAg-positive people
- People born in regions with HBV infection prevalence of ≥2%
- US born people not vaccinated as infants whose parents were born in geographic regions with HBsAg prevalence of ≥8%
- People who inject drugs or have a history of injection drug use

Hepatitis Testing, continued

- People with HIV infection
- Men who have sex with men
- Household contact or former household contacts of people with known HBV infection
- Needle-sharing or sexual contacts of people with known HBV infection
- People on maintenance dialysis, including in-center or home hemodialysis and peritoneal dialysis
- People with elevated liver enzymes

Susceptible people include those who have never been infected with HBV and either did not complete a HepB vaccine series per ACIP recommendations or who are known to be vaccine nonresponders."

The CDC recommends use of the triple panel test which includes hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), and total antibody to hepatitis B core antigen (total anti-HBc). Prior guidance recommended a single test for hepatitis B surface antigen (HBsAg). Any periodic follow up testing can use as appropriate based on the results of the triple panel (CDC, 2023d).

The table below provides CDC recommendations for screening, testing and vaccination for children and adults based on population groups. Infants and Young Adolescents (CDC, 2023c):

Population	Recommendation	
	Screening and Testing	Vaccination
Infants without known hepatitis B exposure	None	Routine vaccination of all infants with the hepatitis B vaccine series, with the first dose administered within 24 hours of birth See https://www.cdc.gov/hepatitis/hbv/vaccchildren.htm
Infants born to hepatitis B surface antigen (HBsAg)-positive pregnant people	See Perinatal Transmission of Hepatitis B virus | CDC	Provide hepatitis B immune globulin (HBIG) and first dose of hepatitis B vaccine within 12 hours of birth, followed by completion of the vaccine series and postvaccination serologic testing See Hepatitis B Vaccination of Infants – Adolescents | CDC See: Management of Infants Born to Women with Hepatitis B Virus Infection for Pediatricians (cdc.gov)
Infants born to pregnant people for whom HBsAg testing results during pregnancy are not available but for whom other evidence suggests maternal HBV infection (e.g., HBV DNA, HBeAg-positive, or pregnant person known to be chronically infected with HBV	See Perinatal Transmission of Hepatitis B virus | CDC	For infants equal to or more than 2,000 grams, provide first dose of hepatitis B vaccine within 12 hours of birth, followed by completion of the vaccine series For infants with birthweight less than 2,000 grams, provide hepatitis B immune globulin (HBIG) and first dose of hepatitis B vaccine within 12 hours of birth, followed by

Hepatitis Testing, continued

Population	Recommendation	
	Screening and Testing	Vaccination
		completion of the vaccine series and postvaccination serologic testing See Hepatitis B Vaccination of Infants – Adolescents | CDC
Adolescents under age 19 years who have not been vaccinated and with no known risk factors	None	Vaccinate See Hepatitis B Vaccination of Infants – Adolescents | CDC

Older Adolescents and Adults (CDC, 2023c):

Population	Recommendation	
	Screening and Testing	Vaccination
Adults with no known risk factors for hepatitis B	If never previously screened, test for HBsAg, anti-HBs, and total anti-HBc (triple panel)	Vaccinate adults aged 18 – 59 years
People with risk factors, regardless of age, such as: – People born in regions of the world with hepatitis B prevalence >2% – U.S.-born people not vaccinated as infants whose parents were born in regions with hepatitis B prevalence >8% – People with current or past injection drug use – People who share needles, or sexual contacts of people with known HBV infection – People currently or formerly incarcerated in a jail, prison, or other detention setting – People with HIV infection – People with current or past hepatitis C virus infection – Men who have sex with men – People with current or past sexually transmitted infections, or multiple sex partners – Current or former household contacts of people with known HBV infection – People on maintenance dialysis, including in-center or home	If never previously screened, test for HBsAg, anti-HBs, and total anti-HBc (triple panel) • *Unless* less than aged 18 years and completed a vaccine series as an infant If previously screened, but still unvaccinated, offer testing to people who have ongoing risk for exposure For additional screening considerations for patients on dialysis, see: Recommendations for Preventing Transmission of Infections Among Chronic Hemodialysis Patients (cdc.gov)	Vaccinate For additional considerations for patients on dialysis, see Recommendations for Preventing Transmission of Infections Among Chronic Hemodialysis Patients (cdc.gov)

Hepatitis Testing, continued

Population	Recommendation	
	Screening and Testing	**Vaccination**
– hemodialysis and peritoneal dialysis, or who are predialysis – People with elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels of unknown origin		
Other populations at risk: – Residents and staff members of facilities for people with developmental disabilities – Health care and public safety personnel with reasonably anticipated risk for exposure to blood or blood-contaminated body fluids – People with diabetes at the discretion of the treating clinician – International travelers to countries with high or intermediate levels of endemic hepatitis B virus infection	If never previously screened, test for HBsAg, anti-HBs, and total anti-HBc (triple panel) • *Unless* aged <18 years and completed a vaccine series as an infant For additional screening considerations for patients on dialysis see: Recommendations for Preventing Transmission of Infections Among Chronic Hemodialysis Patients (cdc.gov)	Vaccinate For additional vaccination considerations for healthcare personnel see: Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices | MMWR (cdc.gov)

Serologic tests for chronic hepatitis B infections should include three HBV seromarkers: HBsAg, anti-HBs, and Total anti-HBc, while testing for acute infection should include HBsAg and IgM anti-HBc. The CDC provides the following chart on interpreting serologic testing results:

Hepatitis Testing, continued

Test and Result	Interpretation	Action
HBsAg—Positive Total anti-HBc — Positive IgM anti-HBc — Positive Anti-HBs — Negative	Acute infection	Link to hepatitis B care
HBsAg — Positive Total anti-HBc — Positive IgM anti-HBc — Negative[1] Anti-HBs — Negative	Chronic Infection	Link to hepatitis B care
HBsAg — Negative Total anti-HBc — Positive Anti-HBs — Positive	Resolved Infection	Counsel about HBV infection reactivation risk
HBsAg — Negative Total anti-HBc — Negative Anti-HBs — Positive[2]	Immune from receipt of prior vaccination (if documented complete series)	If no documentation of full vaccination, then complete vaccine series per ACIP recommendations.
HBsAg — Negative Total anti-HBc — Positive Anti-HBs — Negative	*Only core antibody is positive. See possible interpretations and corresponding actions:*	
	Resolved infection where anti-HBs levels have waned	Counsel about HBV infection reactivation risk
	Occult Infection	Link to hepatitis B care
	Passive transfer of anti-HBc to an infant born to an HBsAg-positive gestational parent	No action
	A false positive, thus patient is susceptible	Offer HepB vaccine per Advisory Committee on Immunization Practices (ACIP)
	A mutant HBsAg strain that is not detectable by laboratory assay	Link to hepatitis B care
HBsAg — Negative Total anti-HBc — Negative Anti-HBs — Negative[3]	Susceptible, never infected (if no documentation of HepB vaccine series completion)	Offer HepB vaccine per ACIP recommendations

[1] IgM anti-HBc also might be positive in persons with chronic infection during severe HBV infection flares or reactivation.

[2] Immune if anti-HBs concentration is >10 mIU/mL after vaccine series completion.

[3] Anti-HBs concentrations might wane over time among vaccine responders. People with a documented, complete HepB vaccine series typically do not need to be revaccinated, except for special populations like patients on hemodialysis or health care personnel.

Figure 1: Interpreting HBV serologic test results (CDC, 2023b)

Hepatitis Testing, continued

For health care providers and viral hepatitis, the CDC makes the following recommendation: "Health care providers should be vaccinated against hepatitis B and tested for hepatitis C after a potential exposure. . . For continued protection, CDC and the Advisory Committee on Immunization Practices (ACIP) recommend that health-care providers and public-safety workers with reasonably anticipated risk for exposures to blood or infectious body fluids receive the complete hepatitis B vaccine series and have their immunity documented through postvaccination testing" (CDC, 2023a).

Hepatitis A

Hepatitis A does not present as a chronic infection, and so the CDC offers no testing recommendations. For acute infections, IgM anti-HAV is the serologic test of choice, but "testing for past acute infection is generally not recommended" (CDC, 2020a).

United States Preventive Services Task Force (USPSTF)

The USPSTF also recommends hepatitis C virus screening in adults aged 18 to 79 years (B recommendation) with anti-HCV antibody testing followed by confirmatory PCR testing (Owens et al., 2020).

The United States Preventive Services Task Force (USPSTF) recommends screening for hepatitis B virus (HBV) infection in adolescents and adults at increased risk for infection. This applies to all asymptomatic, nonpregnant adolescents and adults at increased risk for HBV infection, including those who were vaccinated before being screened for HBV infection. The USPSTF defines some increased-risk groups as "Persons born in the US with parents from regions with higher prevalence are also at increased risk of HBV infection during birth or early childhood, particularly if they do not receive appropriate passive and active immunoprophylaxis (and antiviral therapy for pregnant women with a high viral load)" and also "persons who have injected drugs in the past or currently; men who have sex with men; persons with HIV; and sex partners, needle sharing contacts, and household contacts of persons known to be HBsAg positive" (US Preventive Services Task Force, 2020).

USPSTF recommends the following in relation to screening tests for HBV: "Screening for hepatitis B should be performed with HBsAg tests approved by the US Food and Drug Administration, followed by a confirmatory test for initially reactive results. A positive HBsAg result indicates chronic or acute infection. Serologic panels performed concurrently with or after HBsAg screening allow for diagnosis and to determine further management. (See the Additional Tools and Resources section for serologic test interpretation [Figure 1])."

American Association for the Study of Liver Diseases (AASLD) and the Infectious Disease Society of America (IDSA)

- "One-time, routine, opt out HCV testing is recommended for all individuals aged 18 years and older. Rating: I, B
- One-time HCV testing should be performed for all persons less than 18 years old with activities, exposures, or conditions or circumstances associated with an increased risk of HCV infection (see below). Rating: I,B

Hepatitis Testing, continued

- Prenatal HCV testing as part of routine prenatal care is recommended with each pregnancy. Rating: I, B
- Periodic repeat HCV testing should be offered to all persons with activities, exposures, or conditions or circumstances associated with an increased risk of HCV exposure (see below). Rating: IIa, C
- Annual HCV testing is recommended for all persons who inject drugs, for HIV-infected men who have unprotected sex with men, and men who have sex with men taking pre-exposure prophylaxis (PrEP). Rating: IIa, C
  1. Risk Activities
     - Injection-drug use (current or ever, including those who injected once)
     - Intranasal illicit drug use
     - Use of glass crack pipes
     - Male engagement in sex with men
     - Engagement in chem sex (defined as the intentional combining of sex with the use of particular nonprescription drugs in order to facilitate or enhance the sexual encounter
  2. Risk exposures
     - Persons on long-term hemodialysis (ever)
     - Persons with percutaneous/parenteral exposures in an unregulated setting
     - Healthcare, emergency medical, and public safety workers after needlestick, sharps, or mucosal exposures to HCV-infected blood
     - Children born to HCV-infected women
     - Recipients of a prior transfusion or organ transplant, including persons who:
       - Were notified that they received blood from a donor who later tested positive for HCV
       - Received a transfusion of blood or blood components, or underwent an organ transplant before July 1992
       - Received clotting factor concentrates produced before 1987
     - Persons who were ever incarcerated
  3. Other considerations and circumstances
     - HIV infection
     - Sexually active persons about to start pre-exposure prophylaxis (PreP) for HIV
     - Chronic liver disease and/or chronic hepatitis, including unexplained elevated alanine aminotransferase (ALT) levels
     - Solid organ donors (living and deceased) and solid organ transplant recipients (AASLD-IDSA, 2021)"

Recommendations for Initial HCV Testing and Follow-up

- "HCV-antibody testing with reflex HCV RNA polymerase chain reaction (PCR) is recommended for initial HCV testing. Rating: Class I, Level A
- Among persons with a negative HCV-antibody test who were exposed to HCV within the prior 6 months, HCV-RNA or follow-up HCV-antibody testing 6 months or longer after exposure is

Hepatitis Testing, continued

recommended. HCV-RNA testing can also be considered for immunocompromised persons. Rating: Class I, Level C

- Among persons at risk of reinfection after previous spontaneous or treatment-related viral clearance, initial HCV-RNA testing is recommended because a positive HCV-antibody test is expected. Rating: Class I, Level C
- Persons found to have a positive HCV-antibody test and negative results for HCV RNA by PCR should be informed that they do not have evidence of current (active) HCV infection but are not protected from reinfection. Rating: Class I, Level A
- Quantitative HCV-RNA testing is recommended prior to the initiation of antiviral therapy to document the baseline level of viremia (i.e., baseline viral load).
  Rating: Class I, Level A
- HCV genotype testing may be considered for those in whom it may alter treatment recommendations. Rating: Class I, Level A" (AASLD-IDSA, 2022b; Ghany & Morgan, 2020)

For diagnosing and monitoring acute HCV infections, AASLD-IDSA issued the following recommendations:

- HCV antibody and HCV RNA testing are recommended when acute HCV infection is suspected due to exposure, clinical presentation, or elevated aminotransferase levels (Rating: Class I, Level C)
- "After the initial diagnosis of acute HCV with viremia (defined as quantifiable RNA), HCV treatment should be initiated without awaiting spontaneous resolution."(Rating: Class I, Level B) (AASLD-IDSA, 2022)

For monitoring patients who are starting hepatitis C treatment, are on treatment, or have completed therapy, AASLD-IDSA issued the following recommendations:

- "The following laboratory tests are recommended within 6 months prior to starting DAA (direct-acting antiviral) therapy:
  - Complete blood count (CBC)
  - International normalized ratio (INR)
  - Hepatic function panel (i.e., serum albumin, total and direct bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST], and alkaline phosphatase levels)
  - Estimated glomerular filtration rate (eGFR)
- The following laboratory tests are recommended any time prior to starting DAA therapy:
  - Quantitative HCV RNA (HCV viral load)
  - If a nonpangenotypic DAA will be prescribed, then test for HCV genotype and subtype" (Rating: Class I, Level C)
- "Quantitative HCV viral load testing is recommended 12 or more weeks after completion of therapy to document sustained virologic response (SVR), which is consistent with cure of chronic infection" (Rating: Class I, Level B) AASLD-IDSA, 2022d)

Recommendations for Post-Treatment Follow-Up for Patients in Whom Treatment Failed

Hepatitis Testing, continued

- "Disease progression assessment every 6 to 12 months with a hepatic function panel, complete blood count (CBC), and international normalized ratio (INR) is recommended if patients are not retreated or fail a second or third DAA treatment course." (Rating: Class I, Level C)
- Surveillance for hepatocellular carcinoma with liver ultrasound examination, with or without alpha fetoprotein (AFP), every 6 months is recommended for patients with cirrhosis in accordance with the AASLD guidance on the diagnosis, staging, and management of hepatocellular carcinoma. Rating: Low, Conditional" (AASLD-IDSA, 2022b).

Recommendations for Monitoring HCV-Infected Women During Pregnancy

- "As part of prenatal care, all pregnant women should be tested for HCV infection, ideally at the initial visit." (Rating: IIb, C)
- "HCV RNA and routine liver function tests are recommended at initiation of prenatal care for HCV-antibody–positive pregnant women to assess the risk of mother-to-child transmission (MTCT) and degree of liver disease. (Rating: I, B)
- All pregnant women with HCV infection should receive prenatal and intrapartum care that is appropriate for their individual obstetric risk(s) as there is no currently known intervention to reduce MTCT." (Rating: I, B)
- In HCV-infected pregnant women with pruritus or jaundice, there should be a high index of suspicion for intrahepatic cholestasis of pregnancy (ICP) with subsequent assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bile acids. (Rating: I, B)
- HCV-infected women with cirrhosis should be counseled about the increased risk of adverse maternal and perinatal outcomes. Antenatal and perinatal care should be coordinated with a maternal-fetal medicine (i.e., high-risk pregnancy) obstetrician (Rating: I, B) (AASLD-IDSA, 2022a)."

Assessment of Liver Disease Severity

A section focused on determining the severity of liver diseases associated with an HCV infection is also included as part of the background of these AASLD-IDSA guidelines. The authors state the following:

"The severity of liver disease associated with chronic HCV infection is a key factor in determining the initial and follow-up evaluation of patients. Noninvasive tests using serum biomarkers, elastography, or imaging allow for accurate diagnosis of cirrhosis in most individuals (see pretreatment workup in When and in Whom to Initiate HCV Therapy). Liver biopsy is rarely required but may be considered if other causes of liver disease are suspected.

Noninvasive methods frequently used to estimate liver disease severity include:

- Liver-directed physical exam (normal in most patients)
- Routine blood tests (e.g., ALT, AST, albumin, bilirubin, international normalized ratio [INR], and CBC with platelet count)
- Serum fibrosis marker panels
- Transient elastography
- Liver imaging (e.g., ultrasound or CT scan) (AASLD-IDSA, 2022b)"

Hepatitis Testing, continued

Testing of Perinatally Exposed Children and Siblings of Children with HCV Infection

- All children born to women with acute or chronic hepatitis C should be tested for HCV infection.
- Antibody-based testing is recommended at or after 18 months of age. (I, A)
- Testing with an HCV-RNA assay can be considered in the first year of life, but the optimal timing of such testing is unknown. (IIa, C)
- Testing with an HCV-RNA assay can be considered as early as 2 months of age. (IIa, B)
- Repetitive HCV-RNA testing prior to 18 months of age is not recommended. (III, A)
- Children who are anti-HCV-positive after 18 months of age should be tested with an HCV RNA assay after age 3 to confirm chronic hepatitis C infection. (I, A)
- The siblings of children with vertically acquired chronic hepatitis C should be tested for HCV infection, if born from the same mother. (I, C) (Ghany & Morgan, 2020)

Testing recommendations relating to the monitoring and medical management of children include

- "Routine liver biochemistries at initial diagnosis and at least annually thereafter are recommended to assess for HCV disease progression. (I, C)"
- "Disease severity assessment by routine laboratory testing and physical examination, as well as use of evolving noninvasive modalities (i.e., transient elastography, imaging, or serum fibrosis markers) is recommended for all children with chronic hepatitis C. (I, B)" (Ghany & Morgan, 2020)

American Association for the Study of Liver Diseases (AASLD)

Hepatitis B

The guidance statements surrounding screening for hepatitis B infection is (shown in more detail following declare that

1. Screening should be performed using both HBsAg and anti-HBs.
2. Screening is recommended in all persons born in countries with a HBsAg seroprevalence of ≥2%, U.S.-born persons not vaccinated as infants whose parents were born in regions with high HBV endemicity (≥8%), pregnant women, persons needing immunosuppressive therapy, and the at-risk groups listed in Table 3.
3. Anti-HBs–negative screened persons should be vaccinated.
4. Screening for anti-HBc to determine prior expo-sure is not routinely recommended but is an important test in patients who have HIV infection, who are about to undergo HCV or anti-cancer and other immunosuppressive therapies or renal dialysis, and in donated blood (or, if feasible, organs)(Terrault et al., 2018).

AASLD recommends that the following groups are at high risk for HBV infection and should be screened and immunized if seronegative (Terrault et al., 2018):

Hepatitis Testing, continued

TABLE 3. Groups at High Risk for HBV Infection Who Should Be Screened

- Persons born in regions of high or intermediate HBV endemicity (HBsAg prevalence of ≥2%)
  - Africa (all countries)
  - North, Southeast, East Asia (all countries)
  - Australia and South Pacific (all countries except Australia and New Zealand)
  - Middle East (all countries except Cyprus and Israel)
  - Eastern Europe (all countries except Hungary)
  - Western Europe (Malta, Spain, and indigenous populations of Greenland)
  - North America (Alaskan natives and indigenous populations of Northern Canada)
  - Mexico and Central America (Guatemala and Honduras)
  - South America (Ecuador, Guyana, Suriname, Venezuela, and Amazonian areas)
  - Caribbean (Antigua-Barbuda, Dominica, Grenada, Haiti, Jamaica, Saint Kitts and Nevis, Saint Lucia, and Turks and Caicos Islands)
- U.S.-born persons not vaccinated as an infant whose parents were born in regions with high HBV endemicity (≥8%)*
- Persons who have ever injected drugs*
- Men who have sex with men*
- Persons needing immunosuppressive therapy, including chemotherapy, immunosuppression related to organ transplantation, and immunosuppression for rheumatological or gastroenterologic disorders.
- Individuals with elevated ALT or AST of unknown etiology*
- Donors of blood, plasma, organs, tissues, or semen
- Persons with end-stage renal disease, including predialysis, hemodialysis, peritoneal dialysis, and home dialysis patients*
- All pregnant women
- Infants born to HBsAg-positive mothers*
- Persons with chronic liver disease, e.g., HCV*
- Persons with HIV*
- Household, needle-sharing, and sexual contacts of HBsAg-positive persons*
- Persons who are not in a long-term, mutually monogamous relationship (e.g., >1 sex partner during the previous 6 months)*
- Persons seeking evaluation or treatment for a sexually transmitted disease*
- Health care and public safety workers at risk for occupational exposure to blood or blood-contaminated body fluids*
- Residents and staff of facilities for developmentally disabled persons*
- Travelers to countries with intermediate or high prevalence of HBV infection*
- Persons who are the source of blood or body fluid exposures that might require postexposure prophylaxis
- Inmates of correctional facilities*
- Unvaccinated persons with diabetes who are aged 19 through 59 years (discretion of clinician for unvaccinated adults with diabetes who are aged ≥60 years)*

*Indicates those who should receive hepatitis B vaccine, if seronegative.
Sources: [23,35,36].

(Terrault et al., 2018).

AASLD proposes the use of various screening methods for the diagnose of hepatitis B infection: "HBsAg and antibody to hepatitis B surface antigen (anti-HBs) should be used for screening (Table 4). Alternatively, antibody to hepatitis B core antigen (anti-HBc) can be utilized for screening as long as those who test positive are further tested for both HBsAg and anti-HBs to differentiate current infection from previous HBV exposure. HBV vaccination does not lead to anti-HBc positivity." The interpretations and follow-up steps of the screening results are summarized in their table:

Hepatitis Testing, continued

TABLE 4. Interpretation of Screening Tests for HBV Infection

Screening Test Results					
HBsAg	Anti-HBc	Anti-HBs	Interpretation	Management	Vaccinate?
+	+	–	Chronic hepatitis B	Additional testing and management needed	No
–	+	+	Past HBV infection, resolved	No further management unless immunocompromised or undergoing chemotherapy or immunosuppressive therapy	No
–	+	–	Past HBV infection, resolved or false-positive	HBV DNA testing if immunocompromised patient	Yes, if not from area of intermediate or high endemicity
–	–	+	Immune	No further testing	No
–	–	–	Uninfected and not immune	No further testing	Yes

Hepatitis C

AASLD recommends not repeating hepatitis C viral load testing outside of antiviral therapy, stating that "the results of virologic testing do not change clinical management or outcomes" (AASLD, 2014).

World Health Organization (WHO)

Hepatitis C

Recommendations on screening for HCV infection (WHO, 2017, 2018):

Testing Approach	Recommendations
Focused testing in most affected populations	In all settings (and regardless of whether delivered through facility- or community-based testing), it is recommended that serological testing for HCV antibody (anti-HCV) be offered with linkage to prevention, care and treatment services to the following: • Adults and adolescents from populations most affected by HCV infection (i.e., who are either part of a population with high HCV seroprevalence or who have a history of exposure and/or high-risk behaviors for HCV infection); • Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis (i.e., symptoms, signs, laboratory markers) (strong recommendation, low quality of evidence) **Note**: Periodic re-testing using HCV NAT should be considered for those with ongoing risk of acquisition or reinfection.

Hepatitis Testing, continued

General population testing	In settings with a ≥2% (intermediate) or ≥5% (high) HCV antibody seroprevalence in the general population, it is recommended that all adults have access to and be offered HCV serological testing with linkage to prevention, care and treatment services. General population testing approaches should make use of existing community- or facility-based testing opportunities or programs such as HIV or TB clinics, drug treatment services and antenatal clinics (conditional recommendation, low quality of evidence)
Which serological assay to use	• To test for serological evidence of past or present infection in adults, adolescents and children (>18 months of age), an HCV serological assay (antibody or antibody/antigen) using either a rapid diagnostic test (RDT) or laboratory-based immunoassay formats that meet minimum safety, quality and performance standards (with regard to both analytical and clinical sensitivity and specificity) is recommended. • In settings where there is limited access to laboratory infrastructure and testing, and/or in populations where access to rapid testing would facilitate linkage to care and treatment, RDTs are recommended. (Strong recommendation, low/moderate quality of evidence)

In a guideline pertaining to the screening, care, and treatment of people with chronic hepatitis C infection, the WHO has provided the following recommendations on hepatitis C screening:

- "It is recommended that HCV serology testing be offered to individuals who are part of a population with high HCV seroprevalence or who have a history of HCV risk exposure/behaviour. Strong recommendation, moderate quality of evidence
- It is suggested that nucleic acid testing (NAT) for the detection of HCV ribonucleic acid (RNA) be performed directly following a positive HCV serological test to establish the diagnosis of chronic HCV infection, in addition to NAT for HCV RNA as part of the assessment for starting treatment for HCV infection. - Conditional recommendation, very low quality of evidence (WHO, 2016)."

The WHO also includes a table which shows the populations with a high HCV prevalence or who have a history of HCV risk. The following groups are included:

- "Persons who have received medical or dental interventions in health-care settings where infection control practices are substandard
- Persons who have received blood transfusions prior to the time when serological testing of blood donors for HCV was initiated or in countries where serological testing of blood donations for HCV is not routinely performed
- People who inject drugs (PWID)
- Persons who have had tattoos, body piercing or scarification procedures done where infection control practices are substandard
- Children born to mothers infected with HCV
- Persons with HIV infection

Hepatitis Testing, continued

- Persons who use/have used intranasal drugs
- Prisoners and previously incarcerated persons (WHO, 2016)"

Finally, the WHO mentions liver function tests several times, stating that "A number of clinical considerations are important for the management of persons with chronic HCV infection"; further, "Pre-treatment evaluation of the risk of adverse events should be based on the patient's clinical details, concomitant medications, and knowledge of treatment regimen to be administered. The potential for DDIs [drug-drug interactions] should be assessed before treatment, and a regimen that has a low risk of DDI selected. Standard laboratory tests that are assessed prior to treatment initiation include a full blood count (FBC), international normalized ratio (INR), renal function and liver function tests: ALT, AST, bilirubin, albumin and alkaline phosphatase (WHO, 2016)."

The WHO also mentions that "in persons with HCV infection being treated for TB, it is important to monitor liver function tests" and that "Baseline liver function tests for individuals with chronic liver disease are encouraged prior to initiating treatment for latent TB infection. For individuals with abnormal baseline test results, routine periodic laboratory testing should be carried out during the treatment of latent TB infection (WHO, 2016)."

The recommendations of the WHO for assays and strategies regarding hepatitis C testing are summarized in their table, captured below (WHO, 2017):

HOW TO TEST FOR CHRONIC HCV INFECTION AND MONITOR TREATMENT RESPONSE	
Topic	**Recommendations***
Which serological assays to use	• To test for serological evidence of past or present infection in adults, adolescents and children (>18 months of age[1]), an HCV serological assay (antibody or antibody/antigen) using either RDT or laboratory-based immunoassay formats[2] that meet minimum safety, quality and performance standards[3] (*with regard to both analytical and clinical sensitivity and specificity*) is recommended. - In settings where there is limited access to laboratory infrastructure and testing, and/or in populations where access to rapid testing would facilitate linkage to care and treatment, RDTs are recommended. *Strong recommendation, low/moderate quality of evidence*
Serological testing strategies	In adults and children older than 18 months[1], a single serological assay for initial detection of serological evidence of past or present infection is recommended prior to supplementary nucleic acid testing (NAT) for evidence of viraemic infection. *Conditional recommendation, low quality of evidence*
Detection of viraemic infection	• Directly following a reactive HCV antibody serological test result, the use of quantitative or qualitative NAT for detection of HCV RNA is recommended as the preferred strategy to diagnose viraemic infection. *Strong recommendation, moderate/low quality of evidence* • An assay to detect HCV core (p22) antigen, which has comparable clinical sensitivity to NAT, is an alternative to NAT to diagnose viraemic infection[4]. *Conditional recommendation, moderate quality of evidence*
Assessment of HCV treatment response	• Nucleic acid testing for qualitative or quantitative detection of HCV RNA should be used as test of cure at 12 or 24 weeks (i.e. sustained virological response (SVR12 or SVR24)) after completion of antiviral treatment. *Conditional recommendation, moderate/low quality of evidence*

Hepatitis B

Hepatitis Testing, continued

The below table details the populations who should be tested for chronic hepatitis B infection, according to the WHO (WHO, 2017).

WHO TO TEST FOR CHRONIC HBV INFECTION	
Testing approach and population	**Recommendations***
General population testing	1. In settings with a ≥2% or ≥5%[1] HBsAg seroprevalence in the general population, it is recommended that all adults have routine access to and be offered HBsAg serological testing with linkage to prevention, care and treatment services. General population testing approaches should make use of existing community- or health facility-based testing opportunities or programmes such as at antenatal clinics, HIV or TB clinics. *Conditional recommendation, low quality of evidence*
Routine testing in pregnant women	2. In settings with a ≥2% or ≥5%%[1] HBsAg seroprevalence in the general population, it is recommended that HBsAg serological testing be routinely offered to all pregnant women in antenatal clinics[2], with linkage to prevention, care and treatment services. Couples and partners in antenatal care settings should be offered HBV testing services. *Strong recommendation, low quality of evidence*
Focused testing in most affected populations	*3.* In all settings (and regardless of whether delivered through facility- or community-based testing), it is recommended that HBsAg serological testing and linkage to care and treatment services be offered to the following individuals: • **Adults and adolescents from populations most affected by HBV infection**[3] (i.e. who are either part of a population with high HBV seroprevalence or who have a history of exposure and/or high-risk behaviours for HBV infection); • **Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis**[4] (i.e. symptoms, signs, laboratory markers); • **Sexual partners, children and other family members, and close household contacts** of those with HBV infection[5]; • **Health-care workers:** in all settings, it is recommended that HBsAg serological testing be offered and hepatitis B vaccination given to all health-care workers who have not been vaccinated previously (*adapted from existing guidance on hepatitis B vaccination*[6]) *Strong recommendation, low quality of evidence*
Blood donors *Adapted from existing 2010 WHO guidance (Screening donated blood for transfusion transmissible infections[7])*	4. In all settings, screening of blood donors should be mandatory with linkage to care, counselling and treatment for those who test positive.

Similarly, the recommendations of the WHO for assays and strategies regarding hepatitis B testing are summarized in their table, captured below (WHO, 2017):

Hepatitis Testing, continued

HOW TO TEST FOR CHRONIC HBV INFECTION AND MONITOR TREATMENT RESPONSE	
Topic	**Recommendations***
Which serological assays to use	• For the diagnosis of chronic HBV infection in adults, adolescents and children (>12 months of age[1]), a serological assay (in either RDT or laboratory-based immunoassay format[2]) that meets minimum quality, safety and performance standards[3] (*with regard to both analytical and clinical sensitivity and specificity*) is recommended to detect hepatitis B surface antigen (HBsAg). - In settings where existing laboratory testing is already available and accessible, laboratory-based immunoassays are recommended as the preferred assay format. - In settings where there is limited access to laboratory testing and/or in populations where access to rapid testing would facilitate linkage to care and treatment, use of RDTs is recommended to improve access. *Strong recommendation, low/moderate quality of evidence*
Serological testing strategies	• In settings or populations with an HBsAg seroprevalence of ≥0.4%[4], a single serological assay for detection of HBsAg is recommended, prior to further evaluation for HBV DNA and staging of liver disease. • In settings or populations with a low HBsAg seroprevalence of <0.4%[4], confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT assay for detection of HBsAg may be considered[5]. *Conditional recommendation, low quality of evidence*
Detection of HBV DNA – assessment for treatment *Adapted from existing guidance (WHO HBV 2015 guidelines[6])*	• Directly following a positive HBsAg serological test, the use of quantitative or qualitative nucleic acid testing (NAT) for detection of HBV DNA is recommended as the preferred strategy and to guide who to treat or not treat. *Strong recommendation, moderate/low quality of evidence*
Monitoring for HBV treatment response and disease progression *Existing guidance (WHO HBV 2015 guidelines[6])*	• **It is recommended that the following be monitored at least annually:** - ALT levels (and AST for APRI), HBsAg[7], HBeAg[8], and HBV DNA levels (where HBV DNA testing is available) - Non-invasive tests (APRI score or transient elastography) to assess for presence of cirrhosis in those without cirrhosis at baseline; - If on treatment, adherence should be monitored regularly and at each visit. *Strong recommendation, moderate quality of evidence* **More frequent monitoring is recommended:** • **In persons on treatment or following treatment discontinuation:** more frequent on-treatment monitoring (at least every 3 months for the first year) is indicated in: persons with more advanced disease (compensated or decompensated cirrhosis[9]); during the first year of treatment to assess treatment response and adherence; where treatment adherence is a concern; in HIV-coinfected persons; and in persons after discontinuation of treatment. *Conditional recommendation, very low quality of evidence* • **In persons who do not yet meet the criteria for antiviral therapy:** i.e. persons who have intermittently abnormal ALT levels or HBV DNA levels that fluctuate between 2000 IU/mL and 20 000 IU/mL (where HBV DNA testing is available) and in HIV-coinfected persons[7]. *Conditional recommendation, low quality of evidence*

American Gastroenterological Association (AGA)

Hepatitis B

"The AGA recommends screening for HBV (HBsAg and anti-HBc, followed by a sensitive HBV DNA test if positive) in patients at moderate or high risk who will undergo immunosuppressive drug therapy. (*Strong recommendation; Moderate-quality evidence*) The AGA suggests against routinely screening for HBV in patients who will undergo immunosuppressive drug therapy and are at low risk. (*Weak recommendation; Moderate-quality evidence*) Comments: *Patients in populations with a baseline prevalence likely exceeding 2% for chronic HBV should be screened according to Centers for Disease Control and Prevention and US Preventive Services Task Force recommendations*" (Reddy et al., 2015).

Hepatitis Testing, continued

Hepatitis C

The AGA released best practice statements for care of patients with chronic HCV that have achieved a sustained virologic response (SVR).

- "SVR should be confirmed by undetectable HCV RNA at 12 weeks after completion of an all-oral DAA treatment regimen."
- "Routine confirmation of SVR at 48 weeks post end of treatment is recommended. Testing for HCV RNA at 24 weeks post treatment should be considered on an individual patient basis."
- "Routine testing for HCV RNA beyond 48 weeks after end of treatment to evaluate for late virologic relapse is not supported by available evidence; periodic testing for HCV RNA is recommended for patients with ongoing risk factors for reinfection" (Jacobson et al., 2017).

The AGA has also released a "pathway" for HCV treatment (an algorithm).

Prior to treatment, the AGA recommends identifying the HCV genotype, as well as taking a hepatic function panel (defined as albumin, total and direct bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase).

For all three lengths of treatment courses (8, 12, 16 weeks), the AGA recommends assessing viral load and liver function (the same hepatic panel listed above) (Kanwal et al., 2017).

European Association for the Study of the Liver (EASL)

Hepatitis C

The EASL released guidelines on treatment of hepatitis C. The EASL recommends:

- "Screening strategies for HCV infection should be defined according to the local epidemiology of HCV infection, ideally within the framework of local, regional or national action plans.
- Liver disease severity must be assessed prior to therapy.
- Rapid diagnostic tests using serum, plasma, fingerstick whole blood or crevicular fluid (saliva) as matrices can be used instead of classical EIAs as point-of-care tests to facilitate anti-HCV antibody screening and improve access to care.
- "It is still useful to determine the HCV genotype and subtype where such determination is available and does not limit access to care, to identify patients who may benefit from treatment tailoring. However, "testing for HCV resistance prior to treatment is not recommended" (EASL, 2018, 2020).

Hepatitis B

- The EASL states that "The initial evaluation of a subject with chronic HBV infection should include a complete history, a physical examination, assessment of liver disease activity and severity and markers of HBV infection (Fig. 1). In addition, all first-degree relatives and sexual partners of subjects with chronic HBV infection should be advised to be tested for HBV serological markers (HBsAg, anti-HBs, anti-HBc) and to be vaccinated if they are negative for these markers."
- "The assessment of the severity of liver disease is important to identify patients for treatment and HCC surveillance. It is based on a physical examination and biochemical parameters (aspartate aminotransferase [AST] and ALT, gamma-glutamyl transpeptidase [GGT], alkaline phosphatase,

Hepatitis Testing, continued

bilirubin, and serum albumin and gamma globulins, full blood count and prothrombin time). An abdominal hepatic ultrasound is recommended in all patients. A liver biopsy or a non-invasive test should be performed to determine disease activity in cases where biochemical and HBV markers reveal inconclusive results."

- "HBeAg and anti-HBe detection are essential for the determination of the phase of chronic HBV infection."
- "Measurement of HBV DNA serum level is essential for the diagnosis, establishment of the phase of the infection, the decision to treat and subsequent monitoring of patients."
- "Serum HBsAg quantification can be useful, particularly in HBeAg-negative chronic HBV infection and in patients to be treated with interferon-alfa (IFNα)."
- "HBV genotype is not necessary in the initial evaluation, although it may be useful for selecting patients to be treated with IFNα offerering prognostic information for the probability of response to IFNα therapy and the risk of HCC."
- "Co-morbidities, including alcoholic, autoimmune, metabolic liver disease with steatosis or steatohepatitis and other causes of chronic liver disease should be systematically excluded including co-infections with hepatitis D virus (HDV), hepatitis C virus (HCV) and HIV."
- "Testing for antibodies against hepatitis A virus (anti-HAV) should be performed, and patients with negative anti-HAV should be advised to be vaccinated against HAV."

Indian Health Services (IHS)

Indian Health Services published recommendations on Hepatitis C screening. IHS recommends using an anti-HCV antibody test such as a point-of-care test on a fingerstick capillary or venipuncture whole-blood sample or a laboratory-based HCV ELISA test on a serum sample. IHS recommends screening the following patients:

- "Adults 18 years and older, including people with diabetes, at least once for HCV infection, regardless of their risk factors.
- All pregnant persons, regardless of age, during each pregnancy.
- People at higher risk of HCV exposure (IHS, 2021)."

IHS also provides guidance on how to diagnose a chronic HCV infection:

- "For individuals with a positive HCV antibody screening test result, perform the laboratory-based HCV RNA PCR test to confirm the presence of HCV.
- The presence of HCV indicates active infection. These individuals should be referred for direct acting anti-viral (DAA) agent treatment.
- The absence of HCV indicates no active infection.
- For individuals with a negative HCV antibody test result who might have been exposed to HCV within the previous 6 months, perform an HCV RNA PCR or follow-up HCV antibody test at least 6 months after exposure (IHS, 2021)."

Regarding hepatitis B, the HIS suggests that

"People who inject drugs illicitly, including participants in substance abuse treatment programs, should be offered screening and counseling for chronic HBV infection. Testing should include a serologic assay for hepatitis B surface antigen (HBsAg) offered as a part of routine care, and if the result is positive, be

accompanied by appropriate counseling and referral for recommended clinical evaluation and care. Previous and current sex partners and household and needle-sharing contacts of HBsAg-positive persons should be identified. Unvaccinated sex partners and household and needle-sharing contacts should be tested for HBsAg and for antibody to the hepatitis B core antigen (anti-HBc) or antibody to the hepatitis B surface antigen (anti-HBsAg)" (Indian Health Service).

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
86704	Hepatitis B core antibody (HBcAb); total
86705	Hepatitis B core antibody (HBcAb); IgM antibody
86706	Hepatitis B surface antibody (HBsAb)
86803	Hepatitis C antibody
86804	Hepatitis C antibody; confirmatory test (eg, immunoblot)
87340	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; hepatitis B surface antigen (HBsAg)
87341	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; hepatitis B surface antigen (HBsAg) neutralization
87517	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis B virus, quantification
87520	Infectious agent detection by nucleic acid (dna or rna); hepatitis c, direct probe technique
87521	Infectious agent detection by nucleic acid (dna or rna); hepatitis c, amplified probe technique
87522	Infectious agent detection by nucleic acid (dna or rna); hepatitis c, quantification, includes reverse transcription when performed
87902	Infectious agent genotype analysis by nucleic acid (dna or rna); hepatitis c virus
G0472	Hepatitis C antibody screening, for individual at high risk and other covered indication(s)
G0499	Hepatitis b screening in non-pregnant, high risk individual includes hepatitis b surface antigen (hbsag), antibodies to hbsag (anti-hbs) and antibodies to hepatitis

	b core antigen (anti-hbc), and is followed by a neutralizing confirmatory test, when performed, only for an initially reactive hbsag result

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
8/19/22	Modified overall coverage criteria to align with updated clinical standards.
10/16/23	The following changes were implemented: Changed title of policy to "Hepatitis Testing" (was previously titled, "Hepatitis C); included coverage criteria for Hepatitis B testing, and guidance on HCV testing in pregnant individuals moved from being coverage criteria to being a note in the Policy Description. Also, added CPT codes 86704, 86705, 86706, 87340, 87341, 87517, G0499.
1/8/24	The following changes were implemented: Addition of new coverage criteria #1: "For all individuals 18 years of age and older, triple panel testing (hepatitis B surface antigen [HBsAg], hepatitis B surface antibody [anti-HBs], total antibody to hepatitis B core antigen [anti-HBc]) for Hepatitis B (HBV) infection once per lifetime **MEETS COVERAGE CRITERIA**."; addition of "who have recently traveled to" to coverage criteria #2bi; addition of the following coverage criteria: # 2biii ("For individuals with a history of incarceration", 2bv ("For individuals with a history of sexually transmitted infections or multiple sex partners"), 2bix ("For individuals with an active hepatitis C virus infection or who have a history of hepatitis C virus infection"), and 2bxii ("For individuals with diabetes"); previous coverage criteria #2, now coverage criteria #3, updated to reflect the change in CDC guidance: now that triple panel screening is recommended for all high-risk situations instead of recommendations for 1 to 2 components of that panel, follow up with components of the triple panel screen would not be needed, and coverage criteria #3 now reads: "3) For individuals who test positive for anti-HBc, follow up IgM antibody to anti-HBc (IgM anti-HBc) testing to distinguish between an acute or chronic infection **MEETS COVERAGE CRITERIA**."

HIV Genotyping and Phenotyping

Policy #: AHS – M2093	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 8/24/22 (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

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).

II. Related Policies

Policy Number	Policy Title
AHS-M2116	Plasma HIV-1 and HIV-2 RNA Quantification for HIV Infection

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. HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** in patients who have failed a course of antiviral therapy OR have suboptimal viral load reduction OR have been noncompliant with therapy.
2. HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** for guiding treatment decisions in patients with acute or recent infection (within the last 6 months).

3. HIV genotyping or phenotyping in antiretroviral naive patients entering treatment **MEETS COVERAGE CRITERIA.**
4. HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** for all HIV-infected pregnant individuals in the following situations:
    a. Before initiation of antiretroviral therapy
    b. For those with detectable HIV RNA levels
5. HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** and is required prior to beginning doravirine.
6. HIV phenotyping **MEETS COVERAGE CRITERIA** in treatment-experienced individuals on failing regimens who are thought to have multidrug resistance.

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 prediction using genotypic comparison to known genotypic/phenotypic database **DOES NOT MEET COVERAGE CRITERIA**.

IV. 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 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 a single 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 ~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) (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 (Abbott, 2018). Further, 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).

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. The primary phenotypic assay is "PhenoSense" from LabCorp although "Antivirogram" was used in the past (Kozal, 2019b). 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, 2020).

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 5 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 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).

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).

Taylor et al. (2019) have developed a MiSeq-HyDRA platform for enhanced HIV drug resistance genotyping and surveillance; this platform uses next generation sequencing (NGS) as opposed to Sanger sequencing (SS) methods which are limited due to low data throughput and limited detection of low abundant drug resistant variants (LADRVs). NGS and SS are both DNA sequencing techniques. The authors tested this novel platform with HIV-1 samples amplified at viral loads of ≥1,000 copies/ml. "The gross error rate of this platform was determined at 0.21%, and minor variations were reliably detected

down to 0.50% in plasmid mixtures (Taylor et al., 2019).” The authors conclude by stating that this genotypic platform using NGS has many advantages including an increased sensitivity for LADRV detection, reduced costs and labor, and the potential to routinely monitor for HIV drug resistance.

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).

V. Guidelines and Recommendations

Department of Health and Human Services (DHHS)

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

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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).

HIV Genotyping and Phenotyping, continued

- 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 antiretroviral therapy-naïve persons:
  - 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 (RT) and protease (PR) genes. If transmitted integrase strand transfer inhibitor (INSTI) resistance is a concern, 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:

HIV Genotyping and Phenotyping, continued

- Persons with virologic failure and HIV RNA levels >1,000 copies/mL (AI)
- Persons with HIV RNA levels >500 copies/mL but <1,000 copies/mL, drug-resistance testing may be unsuccessful but should still be considered (BII)
- Persons with suboptimal viral load reduction (AII)

- When a person with HIV experiences virologic failure while receiving an INSTI-based regimen, genotypic testing for INSTI resistance (which may need to be ordered separately) should be performed to determine whether to include a drug from this class in subsequent regimens (AII).
- Drug-resistance testing in the setting of virologic failure should be performed while the person is taking prescribed ARV drugs or, if that is not possible, within 4 weeks after discontinuing therapy (AII). If more than 4 weeks have elapsed since the ARVs 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).
- Genotypic testing is preferred over phenotypic resistance testing to guide therapy in persons with suboptimal virologic response or virologic failure while on first- or second-line regimens and in individuals 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 persons with known or suspected complex drug resistance mutation patterns (BIII).
- All prior and current drug-resistance test results, if available, should be considered when constructing a new regimen for a patient (AIII)."

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 >1,000 copies/mL (AI). In patients with HIV RNA levels >500 copies/mL but <1000 copies/mL, testing may not be successful but should still be considered (BII).

- Resistance testing should be done while the patient is taking ART or, if that is not possible, within 4 weeks after ART discontinuation (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 regimens and for those with noncomplex resistance patterns (AII).
- All prior and current drug-resistance testing results should be reviewed and considered when designing a new regimen for a patient experiencing virologic failure (AIII).
- When virologic failure occurs while a patient is on an INSTI based regimen, 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 HIV-infected pregnant women: Genotypic resistance testing is recommended for all pregnant women 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: 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).

The Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine (ASHM) Sub-Committee for Guidance on HIV Management in Australia

The Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine (ASHM) Sub-Committee for Guidance on HIV Management in Australia has released commentary to the US DHHS Guidelines for the use of Antiretroviral Agents in HIV-1 Infected Adults and Adolescents. The Panel's recommendations are below:

"For Antiretroviral Therapy-Naive Persons:

- 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 When a person with HIV experiences virologic failure while receiving an INSTI-based regimen, genotypic testing for INSTI resistance (which may need to be ordered separately) should be performed to determine whether to include a drug from this class in subsequent regimens (AII).
- Drug-resistance testing in the setting of virologic failure should be performed while the person is taking prescribed ARV drugs or, if that is not possible, within 4 weeks after discontinuing therapy (AII). If more than 4 weeks have elapsed since the ARVs 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).
- Genotypic testing is preferred over phenotypic resistance testing to guide therapy in persons with suboptimal virologic response or virologic failure while on first- or second-line regimens and in individuals 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 persons with known or suspected complex drug-resistance mutation patterns (BIII).
- All prior and current drug-resistance test results, if available, should be considered when constructing a new regimen for a patient (AIII).

  Rating of Recommendations: A = Strong; B = Moderate; C = Optional
  Rating of Evidence: I = Data from randomized controlled trials; II = Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes; III = Expert opinion" (ASHM, 2018).

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, 2021)

International Antiviral Society – USA Panel

The International Antiviral (formerly AIDS) Society-USA expert panel has provided the following recommendations:
"Recommendations for Resistance Testing in Clinical Practice: Who and When to Test"

- HIV resistance testing is recommended for all individuals with HIV infection
  - Who are newly diagnosed and presumably ART-naïve;
    - As soon as an individual is diagnosed with HIV-1 infection
    - In any case, before ART is started (AIIa)
  - Who are on antiretroviral treatment and have plasma HIV RNA that is rising to above 200 copies/mL by confirmed measurements after they have been suppressed to below 50 copies/mL;
    - Preferably while on failing ART (AIIa)
  - Who have not achieved full virus suppression after initiating ART
    - ≥6 months after ART initiation (AIIa)
  - Who have interrupted ART containing an NNRTI with a long half-life (eg, efavirenz); or
    - As soon as virus rebounds above 500 HIV-RNA copies/mL, respectively, before re-initiation of ART (AIIa)
  - Who have a significant increase in viral load in a drug-naïve individual not on treatment
    - After confirmation of increase in plasma viremia
    - Increase of plasma viremia of >0.5$\log_{10}$ within approximately 3-6 months that is confirmed by a second HIV-1 RNA measurement (AIII)."

"Recommendations for Methods for HIV-1 Resistance Testing

- As a first choice, genotypic resistance testing is recommended (evidence rating AIIa).
- Phenotypic resistance testing is recommended, in certain situations:
  - 1. to evaluate HIV susceptibility to new and investigational drugs when drug-resistant mutation patterns have not been fully established (evidence rating AIIa);
  - 2. when genotypic test results are too complex to interpret (evidence rating CIII); or
  - 3. when ART options are highly limited and, as a result, salvage ART must rely on residual susceptibilities to different drugs that are difficult to predict from genotypic data (evidence rating CIII).
- The recommended compartment for drug resistance testing is plasma (evidence rating AII).

- Inclusion of the protease and first half of the reverse transcriptase (up to at least nucleotide 215) is recommended for all genotypic testing (evidence rating BIII).
- Routine InSTI resistance testing in drug-naive individuals is currently not recommended (BIII).
- Baseline InSTI resistance testing is recommended in select patients with evidence of TDR, such as those with nRTI- or multi-class resistance (evidence rating AIII).
- Monitoring of TDR/pretreatment drug resistance to InSTI in selected sites in resource-rich settings and low- and middle-income countries is recommended (evidence rating AIII).
- Sequencing of other regions (C-terminus of reverse transcriptase, gag) or even a near full-length of HIV-1 is not recommended for routine clinical management (evidence rating AIIa).
- Genotypic tropism testing is recommended if a CCR5 antagonist is considered for treatment (evidence rating BIIa).
  Peripheral blood mononuclear cell genotypic resistance testing is recommended in patients with low-level viremia or in patients who are virologically suppressed (evidence rating AIII) (Gunthard et al., 2019)

New York State Department of Health AIDS Institute

Determining HIV Drug Resistance

- "When determining the optimal regimen for achieving viral suppression, clinicians should perform genotypic resistance testing that includes the protease (A2), reverse transcriptase (A2), and integrase genes (B2) at baseline, whether or not ART is being initiated.
  - In patients experiencing treatment failure [a] or incomplete viral suppression; such testing should be performed while patients are still on therapy, but no later than 4 weeks after stopping ART, given the rapid return of wildtype virus. (A2)
  - Perform co-receptor tropism testing prior to initiation of a CCR5 antagonist. (A1)
  - If fusion inhibitor resistance is suspected, that test should be obtained as a supplement to the other genotypic resistance tests. (A2)" (NYSDOH, 2020).

European HIV Drug Resistance Guidelines Panel

Guidelines from the European HIV Drug Resistance Guidelines Panel include the following:
"Postexposure prophylaxis

- Use genotypic information from the index case to guide PEP. If this genotype is not known, do not delay PEP, but if a sample from the index case is available, genotype index case to change or simplify PEP if needed.

Which assay to use

- The panel recommends the use of genotyping in most routine clinical situations. Current genotyping can be performed below a viral load of 1,000 copies/ml.
  Consider additional phenotyping for new drugs, in heavily pretreated patients and for HIV-2 where genotyping is not easily interpretable" (Vandamme et al., 2011).

New York State Department of Health AIDS Institute (NYSDOH)

Determining HIV Drug Resistance

- When determining the optimal regimen for achieving viral suppression, clinicians should perform genotypic resistance testing that includes the protease (A2), reverse transcriptase (A2), and integrase genes (B2) at baseline, whether or not ART is being initiated.
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    - Perform co-receptor tropism testing prior to initiation of a CCR5 antagonist. (A1)
    - If fusion inhibitor resistance is suspected, that test should be obtained as a supplement to the other genotypic resistance tests. (A2) (NYSDOH, 2020)."

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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
87900	Infectious agent drug susceptibility phenotype prediction using regularly updated genotypic bioinformatics

HIV Genotyping and Phenotyping, continued

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 (e.g., integrase, fusion)
0219U	Infectious agent (human immunodeficiency virus), targeted viral next-generation sequence analysis (i.e., 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

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
8/24/22	Modified coverage criteria #4 to align with updated clinical standards and added coverage criteria #5 and #6.

Identification of Microorganisms Using Nucleic Acid Probes

Policy #: AHS – M2097	**Prior Policy Name & Number (as applicable):**
Implementation Date: 9/15/21	**Date of Last Revision:** 2/22/23, 10/26/23 (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

Nucleic acid hybridization technologies utilize complementary properties of the DNA double-helix structures to anneal together DNA fragments from different sources. These techniques are utilized in polymerase chain reaction (PCR) and fluorescent resonance energy transfer (FRET) techniques to identify microorganisms (Khan, 2014).

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.

Identification of Microorganisms using Nucleic Acid Probes, continued

1. The coverage status of nucleic acid identification using direct probe, amplified probe, or quantification for the microorganism's procedure codes is summarized in Table 1 below. "MCC" in the table below indicates that the test **MEETS COVERAGE CRITERIA**; while "DNMCC" tests indicates that the test **DOES NOT MEET COVERAGE CRITERIA**.

Microorganism	Direct Probe	Amplified Probe	Quantification
Bartonella henselae or *quintana*		87471(MCC)	87472 (DNMCC)
Non-vaginal *Candida* species	87480 (DNMCC)	87481 (DNMCC)	87482 (DNMCC)
Chlamydia pneumoniae	87485 (MCC)	87486 (MCC)	87487 (DNMCC)
Clostridium difficile	87493 (MCC)		
Cytomegalovirus	87495 (MCC)	87496 (MCC)	87497 (MCC)
Enterococcus, Vancomycin-resistant (e.g., enterococcus vanA, vanB)		87500 (MCC)	
Enterovirus		87498 (MCC)	
Hepatitis G	87525 (DNMCC)	87526 (DNMCC)	87527 (DNMCC)
Herpes virus-6	87531 (MCC only for individuals < 5 y/o, solid organ/bone marrow transplant, or HIV patient) 87531 (DNMCC in all other situations)	87532 (DNMCC)	87533 (MCC only for individuals < 5 y/o, solid organ/bone marrow transplant, or HIV patient) 87533 (DNMCC in all other situations)
Legionella pneumophila	87540 (MCC)	87541 (MCC)	87542 (DNMCC)
Orthopoxvirus		87593 (MCC)	
Mycoplasma pneumoniae	87580 (MCC)	87581 (MCC)	87582 (DNMCC)
Mycoplasma genitalium		87563 (MCC)	
Respiratory syncytial virus		87634 (MCC)	
Staphylococcus aureus		87640 (MCC)	
Staphylococcus aureus, methicillin resistant		87641 (MCC)	

2. 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**.

III. Scientific Background

Nucleic acid hybridization technologies, including polymerase chain reaction (PCR), ligase- or helicase-dependent amplification, and transcription-mediated amplification, are beneficial tools for pathogen detection in blood culture and other clinical specimens due to high specificity and sensitivity (Khan, 2014). The use of nucleic acid-based methods to detect bacterial pathogens in a clinical laboratory setting offers "increased sensitivity and specificity over traditional microbiological techniques" due to its specificity, sensitivity, reduction in time, and high-throughput capability; however, "contamination

potential, lack of standardization or validation for some assays, complex interpretation of results, and increased cost are possible limitations of these tests" (Mothershed & Whitney, 2006).

IV. Guidelines and Recommendations

World Health Organization (WHO)

For detection of monkeypox, the WHO recommends "detection of viral DNA by polymerase chain reaction (PCR)" as the preferred laboratory test and recommends that any individual with a suspected case should be offered testing. They note that the best specimens for diagnosis are taken directly from the rash. Antigen and antibody detection may not be able to distinguish between orthopoxviruses (WHO, 2022).

2018 Infectious Diseases Society of America (IDSA)

Specific guidelines for testing of many organisms listed within the policy coverage criteria is found in the updated 2018 Infectious Diseases Society of America (IDSA**)** guidelines and recommendations titled, "A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology" (Miller et al., 2018). "This document is organized by body system, although many organisms are capable of causing disease in >1 body system. There may be a redundant mention of some organisms because of their propensity to infect multiple sites. One of the unique features of this document is its ability to assist clinicians who have specific suspicions regarding possible etiologic agents causing a specific type of disease. When the term "clinician" is used throughout the document, it also includes other licensed, advanced practice providers. Another unique feature is that in most chapters, there are targeted recommendations and precautions regarding selecting and collecting specimens for analysis for a disease process. It is very easy to access critical information about a specific body site just by consulting the table of contents. Within each chapter, there is a table describing the specimen needs regarding a variety of etiologic agents that one may suspect as causing the illness. The test methods in the tables are listed in priority order according to the recommendations of the authors and reviewers (Miller et al., 2018)."

Centers of Disease Control and Prevention (CDC)

Candida Auris (C. auris)

The CDC writes that "Molecular methods based on sequencing the D1-D2 region of the 28s rDNA or the Internal Transcribed Region (ITS) of rDNA also can identify *C. auris*." The CDC further notes that various PCR methods have been developed for identifying *C. auris* (CDC, 2020a).

Chlamydia Pneumoniae (C. pneumoniae)

The CDC writes that RT-PCR is the "preferred" method of detecting a *C. pneumoniae* infection, with qPCR preferred for an acute infection. The CDC further notes that a positive culture should be confirmed by a second test, such as PCR (CDC, 2021a).

Ebola

Identification of Microorganisms using Nucleic Acid Probes, continued

The CDC states that for diagnosis of Ebola, "there must be a combination of symptoms suggestive of EVD AND a possible exposure to EVD within 21 days before the onset of symptoms". The CDC notes that PCR is one of the most common diagnostic methods (CDC, 2019a). Such exposures include:

- blood or body fluids from a person sick with or who died from EVD,
- objects contaminated with blood or body fluids of a person sick with or who died from EVD,
- infected fruit bats and nonhuman primates (apes or monkeys), or
- semen from an individual who has recovered from EVD.

The CDC notes that PCR is one of the most common diagnostic methods, but also cautions that "When the virus is no longer present in great enough numbers in a patient's blood, PCR methods will no longer be effective. Other methods, based on the detection of antibodies an EVD case produces to an infection, can then be used to confirm a patient's exposure and infection by Ebola virus" (CDC, 2022c).

Giardia

The CDC states that microscopy with direct fluorescent antibody testing (DFA) is considered the test of choice for diagnosing giardiasis, but rapid immunochromatographic cartridge assays, enzyme immunoassay kits, microscopy with trichrome staining, and molecular assays may be alternatively used as well. To obtain more accurate test results, the CDC recommends collecting three stool specimens from patients over the course of a few days. But, only molecular testing (e.g., DNA sequencing) can identify *Giardia* strains (CDC, 2021b).

Monkeypox Virus

The CDC defines a suspect case of monkeypox as a "new characteristic rash, or meets one of the epidemiologic criteria and has a high clinical suspicion for monkeypox." A probable case is defined as "no suspicion of other recent Orthopoxvirus exposure (e.g., Vaccinia virus in ACAM2000 vaccination) AND demonstration of the presence of Orthopoxvirus DNA by polymerase chain reaction of a clinical specimen OR Orthopoxvirus using immunohistochemical or electron microscopy testing methods OR Demonstration of detectable levels of anti-orthopoxvirus IgM antibody during the period of 4 to 56 days after rash onset." A confirmed case of monkeypox is defined as "demonstration of the presence of Monkeypox virus DNA by polymerase chain reaction testing or Next-Generation sequencing of a clinical specimen OR isolation of Monkeypox virus in culture from a clinical specimen" (CDC, 2022b).

MRSA

The CDC remarks that nucleic acid amplification tests (NAATs, such as PCR) "can be used for direct detection of mecA, the most common gene mediating oxacillin resistance in staphylococci," but will not detect novel resistance mechanisms or uncommon phenotypes (CDC, 2019a).

Mycoplasma Genitalium

The CDC writes that "Men with recurrent NGU [nongonococcal urethritis] should be tested for *M. genitalium* using an FDA-cleared NAAT. If resistance testing is available, it should be performed and the

results used to guide therapy. Women with recurrent cervicitis should be tested for *M. genitalium*, and testing should be considered among women with PID [pelvic inflammatory disease]. Testing should be accompanied with resistance testing, if available. Screening of asymptomatic M. genitalium infection among women and men or extragenital testing for M. genitalium is not recommended. In clinical practice, if testing is unavailable, M. genitalium should be suspected in cases of persistent or recurrent urethritis or cervicitis and considered for PID" (CDC, 2021d).

Non-Polio Enterovirus

The CDC remarks that their laboratories "routinely" perform qualitative testing for enteroviruses, parechoviruses, and uncommon picornaviruses (CDC, 2018).

Respiratory Syncytial Virus (RSV)

The CDC writes that real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) and antigen detection tests are the most commonly used diagnostic tests and are effective in infants and young children. However, the highly sensitive rRT-PCR is recommended to be used when testing older children and adults with RSV. (CDC, 2022d).

Salmonella

The CDC writes that diagnosis requires detection of the *Salmonella* bacteria, be it through culture or a "culture-independent diagnostic test (CIDT)" (CDC, 2019b).

Miscellaneous

The CDC does not mention the need to quantify [through PCR] *Bartonella*, *Legionella pneumophila* or *Mycoplasma pneumoniae.* However, PCR can be performed for both *Legionella pneumophila* and *Mycoplasma pneumonia*e specimen (CDC, 2020b, 2021c, 2022a). No guidance was found on Hepatitis G.

Committee on Infectious Diseases, American Academy of Pediatrics, 31st Edition (2018-2021, Red Book)

The Committee on Infectious Diseases released joint guidelines with the American Academy of Pediatrics. In it, they note that "the presumptive diagnosis of mucocutaneous candidiasis or thrush usually can be made clinically". They also state that FISH probes may rapidly detect *Candida* species from positive blood culture samples, although PCR assays have also been developed for this purpose (AAP Committee on Infectious Diseases, 2018).

European Centre for Disease Prevention and Control (ECDC)

On May 23, 2022, the ECDC released a rapid risk assessment of the monkeypox multi-country outbreak. They recommend that patients with probable cases should be tested with a "monkeypox virus specific PCR or an orthopoxvirus specific PCR assay which is then confirmed through sequencing" (ECDC, 2022b).

On June 2, 2022, ECDC released interim advice on risk communication and community engagement during the 2022 monkeypox outbreak in Europe. This is a joint report with the WHO regional office for Europe.

They recommend speaking to your doctor about getting tested for monkeypox if you develop a rash with a fever or feeling of discomfort or illness (ECDC, 2022a).

United Kingdom Heath Security Agency (UKHSA)

The UKHSA states that "Mpox is diagnosed by PCR test for the monkeypox virus (MPXV) on a viral swab taken from one or more vesicles or ulcers." Specifically, it is recommended that healthcare workers"Take a viral swab in viral culture medium or viral transport medium (for example Virocult®) from an open sore or from the surface of a vesicle. If other wounds are present, ensure that the sample is definitely taken from a vesicle, an ulcer or a crusted vesicle. Rub the swab over the lesion and place the swab in the collection tube. If there are pharyngeal lesions, a throat swab should also be taken" (UKHSA, 2023). UKHSA also suggests that "A viral throat swab can be taken for high-risk contacts of a confirmed or highly probable case who have developed systemic symptoms but do not have a rash or lesions that can be sampled. Please note that even if the throat swab is negative, the individual must continue with monitoring and isolation as instructed by their local health protection team, and should be reassessed and sampled if further symptoms develop". Lastly, "If follow-up testing is required from a confirmed or highly probable case, either because of clinical deterioration or to inform discharge from isolation to an inpatient setting, additional samples should be taken and should include the following:

- a lesion swab and throat swab in viral transport medium
- a blood sample in an EDTA tube
- a urine sample in a universal sterile container" (UKHSA, 2023).

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.

As of 08/02/2022, a list of current U.S. Food and Drug Administration (FDA, 2022) approved or cleared nucleic acid-based microbial tests is available at: https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests.

VI. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
87471	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, amplified probe technique
87472	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, quantification

Identification of Microorganisms using Nucleic Acid Probes, continued

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
87485	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, direct probe technique
87486	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, amplified probe technique
87487	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, quantification
87493	Infectious agent detection by nucleic acid (DNA or RNA); Clostridium difficile, toxin gene(s), amplified probe technique
87495	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, direct probe technique
87496	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, amplified probe technique
87497	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, quantification
87498	Infectious agent detection by nucleic acid (DNA or RNA); enterovirus, amplified probe technique, includes reverse transcription when performed
87500	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin resistance (e.g., enterococcus species van A, van B), amplified probe technique
87525	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, direct probe technique
87526	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, amplified probe technique
87527	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, quantification
87531	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, direct probe technique
87532	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, amplified probe technique
87533	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, quantification
87540	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, direct probe technique
87541	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, amplified probe technique
87542	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, quantification
87563	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma genitalium, amplified probe technique

Identification of Microorganisms using Nucleic Acid Probes, continued

87580	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, direct probe technique
87581	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, amplified probe technique
87582	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, quantification
87593	Infectious agent detection by nucleic acid (DNA or RNA); orthopoxvirus (eg, monkeypox virus, cowpox virus, vaccinia virus), amplified probe technique, each
87634	Infectious agent detection by nucleic acid (DNA or RNA); respiratory syncytial virus, amplified probe technique
87640	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, amplified probe technique
87641	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, methicillin resistant, 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.

VII. Evidence-based Scientific References

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Identification of Microorganisms using Nucleic Acid Probes, continued

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VIII. Revision History

Revision Date	Summary of Changes
2/22/23	Modified wording in coverage criteria #2 and #3 for clarity. Also, added CPT 87593 (Infectious agent detection by nucleic acid (DNA or RNA); orthopoxvirus (eg, monkeypox virus, cowpox virus, vaccinia virus), amplified probe technique, each), which is a covered code with Select Health.
9/21/23	Removed former coverage criteria #3: "For any other microorganism without a specific CPT code, PCR testing **MEETS COVERAGE CRITERIA.**"

Immune Cell Function Assay

Policy #: AHS – G2098	**Prior Policy Name & Number (as applicable):** AHS – G2098 – Immune Cell Function for Management of Organ Transplant Rejection Assay
Implementation Date: 9/15/21	**Date of Last Revision:** 4/26/22 (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

Immune cell function assays involve measurement of peripheral blood lymphocyte response (intracellular ATP levels, proliferation) following stimulation to assess the degree of functionality of the cell-mediated immune response (Buttgereit, Burmester, & Brand, 2000).

For guidance on procedures utilizing flow cytometry, please refer to AHS-F2019 Flow Cytometry.

II. Related Policies

Policy Number	Policy Title
AHS-F2019	Flow Cytometry
AHS-M2091	Transplant Rejection 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. An immune cell function assay **DOES NOT MEET COVERAGE CRITERIA** for all indications including, but not limited to:
    a. The management of solid organ transplant rejection in an individual undergoing immunosuppressive therapy;
    b. Identification of risk for rejection prior to any solid organ transplantation;
    c. The management of autologous or allogeneic hematopoietic stem cell transplantation;
    d. The management of immunodeficiency disorders including human immunodeficiency virus (HIV) and severe combined immunodeficiency disease (SCID);
    e. The management of or prediction of infection risk in immune mediated disorders including rheumatoid arthritis (RA), multiple sclerosis, and lupus nephritis;
    f. Testing for urticaria;
    g. Diagnosis and management of Lyme disease (for example, iSpot Lyme Test).
    h. Management of inflammatory bowel diseases;
    i. Monitoring immune response following surgery.

IV. Scientific Background

Primary immunodeficiencies are a group of rare disorders in which part of the body's immune system is absent or functions incorrectly. These disorders occur in as many as 1:2000 live births and are most often categorized according to a combination of mechanistic and clinical descriptive characteristics (Bonilla et al., 2015). Specific cellular immunity is mediated by T cells, and defects affecting these T cells underlie the most severe immunodeficiencies. As antibody production by B cells requires intact T cell function, most T cell defects lead to combined (cellular and humoral) immunodeficiency (Butte & Stiehm, 2019).

In vitro studies of T cell function measure peripheral blood T cell responses to several different types of stimuli (Bonilla, 2008):

- Mitogens (such as the plant lectins phytohemagglutinin, concanavalin A, pokeweed mitogen, anti-CD3).
- Specific antigens (such as tetanus and diphtheria toxoids or *Candida albicans* antigens).
- Allogeneic lymphocytes (i.e., mixed lymphocyte culture).

Exposure of T cells to stimulus leads to their metabolic activation and polyclonal expansion (Fernandez-Ruiz et al., 2014). Response can be measured by indicators of proliferation, ATP synthesis and release, or expansion of specific subpopulations (Stiehm, 2017).

The evaluation of specific immune responses is essential for diagnosis of primary immune deficiencies. Screening tests used to evaluate patients with suspected primary immune deficiencies are relatively inexpensive, performed rapidly, and reasonably sensitive and specific (Notarangelo, 2010; Oliveira & Fleisher, 2010). Abnormal screening test results indicate the need for more sophisticated tests. This stepwise approach ensures an efficient and thorough evaluation of mechanisms of immune dysfunction that underlie the clinical presentation; this process includes the narrowing of diagnostic options before using costly sophisticated tests that might be required to arrive at specific diagnoses (Bonilla et al., 2015). Abnormal T cell counts measure T cell mitogen responses that are absent or extremely low; this is a crucial element in the diagnosis of several primary immune deficiencies, most notably, severe combined immunodeficiency (SCID) (Picard et al., 2015). Additionally, T-cell recognition of alloantigens is the primary and central event that leads to the cascade of events that result in rejection of a transplanted organ (Vella, 2020). Several commercial assays have been developed based on the traditional assessment of T-cell stimulation to predict or assess transplant rejection.

The ImmuKnow assay measures the ability of CD4 T-cells to respond to mitogenic stimulation by phytohemagglutinin-L in vitro by quantifying the amount of adenosine triphosphate (ATP) produced and released from these cells following stimulation (Zhang et al., 2016). Since the CD4 lymphocytes orchestrate cell-mediated immunity responses through immunoregulatory signaling, measurement of intracellular ATP levels following CD4 activation is intended to estimate the net state of immune system in immunocompromised patients (Chon, 2021) and one of the few well-established strategies for functional immune monitoring in solid organ transplant recipients (Sottong et al., 2000).

The Pleximmune™ blood test measures the inflammatory immune response of recipient T-cells to the donor in co-culture of lymphocytes from both sources (Ashokkumar et al., 2009; Ashokkumar et al., 2017; Sindhi et al., 2016). The Pleximmune test sensitivity and specificity for predicting acute cellular rejection was found to be 84% and 81%, respectively, in a training set–validation set testing of 214 children. Early clinical experience shows that test predictions are particularly useful in planning immunosuppression in the setting of indeterminate biopsy findings or in modifying protocol-mandated treatment when combined with all other available clinical information about an individual patient (Sindhi et al., 2016).

Clinical Utility and Validity

A population-based study comparing the assay results in healthy controls and solid organ transplant recipients established three categories to define patient's cell-mediated immune response: strong ($\geq$525 ng ml^{-1}), moderate (226–524 ng ml^{-1}) and low ($\leq$225 ng ml^{-1}) (Fernandez-Ruiz et al., 2014; Kowalski et al., 2006). Numerous authors have analyzed the predictive value of the ImmuKnow® (Viracor) assay for acute rejection, as recently summarized in a meta-analysis that found a relatively high specificity (0.75) but a low sensitivity (0.43), with significant heterogeneity across studies (Fernandez-

Immune Cell Function Assay, continued

Ruiz et al., 2014; Ling et al., 2012). The ImmuKnow® assay has been examined in clinical trials for its potential use in monitoring immunosuppression medication regimens in solid organ transplant patients.

Kowalski et al. (2006) performed a meta-analysis of 504 solid organ transplant recipients (heart, kidney, kidney-pancreas, liver, and small bowel) from 10 U.S. centers. The authors found that "A recipient with an immune response value of 25 ng/ml adenosine triphosphate (ATP) was 12 times more likely to develop an infection than a recipient with a stronger immune response. Similarly, a recipient with an immune response of 700 ng/ml ATP was 30 times more likely to develop a cellular rejection than a recipient with a lower immune response value (Kowalski et al., 2006)." The authors also hypothesized an "immunological target of immune function," created by the intersection of odds ratio curves at 280 ng/ml ATP. The authors concluded "the Cylex ImmuKnow assay has a high negative predictive value and provides a target immunological response zone for minimizing risk and managing patients to stability (Kowalski et al., 2006)."

Wang et al. (2014) performed a meta-analysis of six studies which found "The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) of ImmuKnow for predicting the risk of infection were 0.51, 0.75, 1.97, 0.67, and 3.56, respectively. A DOR of 13.81, with a sensitivity of 0.51, a specificity of 0.90, a PLR of 4.45, and an NLR of 0.35, was found in the analysis of the predictive value for acute rejection." The authors concluded, "Our analysis did not support the use of the ImmuKnow assay to predict or monitor the risks of infection and acute rejection in renal transplant recipients. Further studies are needed to confirm the relationships between the ImmuKnow assay and infection and acute rejection in kidney transplantation (Wang et al., 2014)."

Jo et al. (2015) analyzed CD4 T-lymphocytes ATP levels along with lymphocyte subsets in 160 samples from 111 post-allogeneic hematopoietic stem cell transplantation (alloHSCT) patients. In patients with stable status, the 6-month post-alloHSCT ImmuKnow® levels were found to be significantly higher than those tested within 6 months post-alloHSCT. ImmuKnow® results 6 months post-alloHSCT showed low positive correlation with natural killer cell count (r = 0.328) and the values tested later than 6 months post-alloHSCT were positively correlated with CD4 T cell count (r = 0.425). However, ImmuKnow® levels for acute graft-versus-host disease (GVHD) or infection episodes were not significantly different compared to those for stable alloHSCT. The authors concluded that "the combined test of ImmuKnow levels and lymphocyte subsets may be helpful for immune monitoring following alloHSCT."

Ravaioli et al. (2015) aimed to "assess the clinical benefits of adjusting immunosuppressive therapy in liver recipients based on immune function assay results." A total of 100 patients received serial immune function testing via the ImmuKnow in vitro diagnostic assay (compared to 102 controls who received standard practice). The authors found that "based on immune function values, tacrolimus doses were reduced 25% when values were less than 130 ng/mL adenosine triphosphate (low immune cell response) and increased 25% when values were greater than 450 ng/mL adenosine triphosphate (strong immune cell response)" (Ravaioli et al., 2015). The authors also found that survival and infection rates were better in the treatment arm compared to the control arm. Overall, the investigators concluded

Immune Cell Function Assay, continued

"Immune function testing provided additional data which helped optimize immunosuppression and improve patient outcomes" (Ravaioli et al., 2015).

Piloni et al. (2016) evaluated 61 lung recipients who underwent follow-up for lung transplantation between 2010 and 2014 in order to correlate ImmuKnow® values with functional immunity in lung transplant recipients. The authors found that 71 out of 127 samples (56%) showed an over-immunosuppression with an ImmuKnow® assay mean level of 112.92 ng/ml (SD ± 58.2) vs. 406.14 ng/ml (SD ± 167.7) of the rest of our cohort. In the over-immunosuppression group, the authors found 51 episodes of infection (71%). The mean absolute ATP level was significantly different between patients with or without infection (202.38 ± 139.06 ng/ml vs. 315.51 ± 221.60 ng/ml). The authors concluded that "the ImmuKnow assay levels were significantly lower in infected lung transplant recipients compared with non-infected recipients and in RAS patients" (Piloni et al., 2016).

Chiereghin et al. (2017) evaluated symptomatic infectious episodes that occurred during the first year after an organ transplant. A total of 135 infectious episodes were studied with 77 of the infections bacterial, 45 viral, and 13 fungal. Significantly lower median ImmuKnow® intracellular ATP levels were identified in patients with bacterial or fungal infections compared to infection-free patients, whereas patients with viral infection did not have a significantly different median ATP level compared to non-infected patients. The authors concluded that bacteria were responsible for most symptomatic infections post-transplant and that ImmuKnow measurements may be useful for "identifying patients at high risk of developing infection, particularly of fungal and bacterial etiology" (Chiereghin et al., 2017).

Liu et al. (2019) studied the potential of the ImmuKnow assay to diagnose infection in pediatric patients who have received a living-donor liver transplant. A total of 66 patients participated in this study and were divided into infection (n=28) and non-infection (n=38) groups. The researchers report that the "CD4+ T lymphocyte ATP value of the infection group was significantly lower compared with that of the non-infection group" (Liu et al., 2019). This suggests that for pediatric patients who have received a living-donor liver transplant, low CD4+ T lymphocyte ATP levels may be related to infection rates. The ImmuKnow assay may be a helpful tool in this scenario to predict infection.

Weston et al. (2020) used the ImmuKnow assay to adjust immunosuppression in heart transplant recipients with severe systemic infections. In particular, if a patient developed an infection, the ImmuKnow assay was used to recommend adjustments in immunosuppression. This assay was used on 80 patients; thirteen of these patients developed a more serious infection. The researchers conclude that "Heart transplant recipients with severe systemic infections presented with a decreased ImmuKnow®, suggesting over immunosuppression. ImmuKnow® can be used as an objective measurement in withdrawing immunosuppression in heart transplant recipients with severe systemic infections (Weston et al., 2020)."

Ashokkumar et al. (2017) evaluated PlexImmune through the assessment of CD-154 T-cytotoxic memory cells. A total of 280 samples (158 training set, 122 validation) from 214 children were examined. Recipient CD-154 cells induced by stimulation with donor cells were expressed as a fraction of those induced by human leukocyte antigen (HLA) nonidentical cells, and a resulting immunoreactivity index (IR) ≥1 implied increased rejection-risk. The authors found that "an IR of 1.1 or greater in posttransplant

training samples and IR of 1.23 or greater in pretransplant training samples predicted liver transplant (LTx) or intestine transplant (ITx) rejection with sensitivity, specificity, positive, and negative predictive values of 84%, 80%, 64%, and 92%, respectively, and 57%, 89%, 78%, and 74%, respectively (Ashokkumar et al., 2017)." The authors concluded that "Allospecific CD154+T-cytotoxic memory cells predict acute cellular rejection after LTx or ITx in children. Adjunctive use can enhance clinical outcomes (Ashokkumar et al., 2017)."

However, at the present time, there is no consensus on the utility of these tests, despite the amount of literature devoted to determine its real value for predicting post-transplant complications (Clark & Cotler, 2020; Fernandez-Ruiz et al., 2014; Kowalski et al., 2006; Ling et al., 2012; Rodrigo et al., 2012).

Monforte et al. (2021) studied the prognostic value of ImmuKnow® for predicting non-cytomegalovirus (CMV) infections in lung transplant patients. 92 patients were followed for 6 to 12 months after their lung transplant and the assay was carried out at 6, 8, 10, and 12 months. 25% of the patients developed non-CMV infections between 6-12 months after the transplant. At 6 months, 15.2% of patients had a moderate immune response and 84.8% of patients had a low immune response to the infection. In the following 6 months, only one of the patients with a moderate immune response developed a non-CMV infection compared to the 28.2% of low immune response patients who developed a non-CMV infection. The ImmuKnow® assay had a sensitivity of 95.7%, specificity of 18.8%, positive predictive value (PPV) of 28.2%, and negative predictive value (NPV) of 92.9% in detecting a non-CMV infection. The authors conclude that "although ImmuKnow® does not seem useful to predict non-CMV infection, it could identify patients with a very low risk and help us define a target for an optimal immunosuppression" (Monforte et al., 2021).

In an open-label prospective cohort study, Xue et al. (2021) studied the use of the Cylex immune cell function assay for diagnosis of infection after liver transplant in pediatric patients. 216 infants with liver transplants were followed and Cylex ATP values were measured before and after the liver transplant at weeks 1, 2, 3, 4, 8, 12 and 24. After surgery, 74.1% of the transplant patients had a diagnosed infection, 20.4% were clinically stable, and 5.6% experienced acute rejection. The median Cylex ATP value in infant PLTs post-surgery reduced significantly in the infection group compared to stable group. ROC curve analysis determined that the cut-off value of Cylex ATP was 152 ng/mL for diagnosis of infection. The authors conclude "In this study, we demonstrated that low Cylex ATP represented partly over-immunosuppression and had diagnostic value in infant PLTs with infections, which might assist individualized immunosuppression in PLT patients" (Xue et al., 2021).

V. Guidelines and Recommendations

The American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology (ACAAI)

The American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology (ACAAI) published practice parameters for the diagnosis and management of primary immunodeficiency (Bonilla et al., 2015) which stated that:

"Evaluation of specific immune responses is essential for diagnosis of PIDDs [primary immunodeficiency diseases]. Measurement of serum immunoglobulin levels and lymphocyte responses to mitogens are useful indicators of global B- and T-cell development and function."

The guideline also lists "In vitro proliferative response to mitogens and antigens" as an advanced test used when "Abnormal screening test results indicate the need for more sophisticated tests" (Bonilla et al., 2015). The screening test indicated is: flow cytometry to enumerate CD4 and CD8 T cells and NK cells.

Normal or abnormal T cell response to mitogen stimulation is listed in the diagnostic algorithm for the diagnosis of combined or syndromic immunodeficiencies. Specifically, it states that "Infants with low TREC counts should have secondary screening by using flow cytometry to enumerate T-cell numbers and the proportion of naive cells. T-cell counts of less than 1500/mm^3 or a proportion of naive cells of less than 50% should be followed up measuring the in vitro response to a mitogen, such as PHA." It is also listed as a characteristic laboratory finding for WAS, AT related disorders, Good syndrome, XLP1, MSMD, MyD88, WHIM, EV and in the management of DGS, and immuno-osseous dysplasias.

The International Society of Heart and Lung Transplantation

Guidelines for the care of heart transplant recipients published in 2010 by The International Society of Heart and Lung Transplantation do not include ImmuKnow®.

An ISHLT consensus document for the management of antibodies in a heart transplantation was published in 2018. This document do not mention the ImmuKnow or Pleximmune assays, but does state that "Solid-phase assays, such as the Luminex SAB assay, are recommended to detect circulating antibodies" (Kobashigawa et al., 2018).

An ISHLT consensus document for the antibody-mediated rejection of the lung was published in 2016. This consensus document does not mention the ImmuKnow or Pleximmune assays (Levine et al., 2016).

The American Society of Transplantation (AST)

The American Society of Transplantation does not include the use of the ImmuKnow assay in its publication: "Recommendations for Screening, Monitoring and Reporting of Infectious Complications in Immunosuppression Trials in Recipients of Organ Transplantation" (Humar & Michaels, 2006).

Educational guidelines for the management of kidney transplant recipients in the community setting and for infectious diseases in transplant recipients published in 2009 by the American Society of Transplantation (AST) also do not include ImmuKnow® (AST, 2009).

Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation

The International Cytomegalovirus CMV Consensus Group of the Transplantation Society published an international consensus statement on the management of CMV in solid organ transplant in 2018. In it, they note that "Clinical utility studies demonstrate that alteration of patient management based on the results of an immune-based assay is feasible, safe, and cost-effective" (Kotton et al., 2018).

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Immune Cell Function Assay, continued

ImmuKnow® (Viracor, previously, Cylex) is an immune cell function assay cleared for marketing by the U.S. Food and Drug Administration (FDA) in April 2002 to detect cell-mediated immunity (CMI) in an immunosuppressed patient population. Cylex obtained 510(k) clearances from the FDA to market the Immune Cell Function Assay based on substantial equivalence to two flow cytometry reagents. The FDA-indicated use of the Cylex Immune Cell Function Assay is for the detection of cell-mediated immunity in an immunosuppressed population. A subsequent 510(k) marketing clearance for a device modification was issued by the FDA for this assay in 2010. There were no changes to the indications or intended use.

In August 2014, Pleximmune™ (Plexision, Pittsburgh, PA) was approved by FDA through the humanitarian device exemption process. The test is intended for use in the pre-transplantation and early and late post-transplantation period in pediatric liver and small bowel transplant patients for the purpose of predicting the risk of transplant rejection within 60 days after transplantation or 60 days after sampling.

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
86352	Cellular function assay involving stimulation (e.g., mitogen or antigen) & detection of biomarker (e.g., ATP)
0018M	Transplantation medicine (allograft rejection, renal), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score Proprietary test: Pleximark Lab/Manufacturer: Plexision, 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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