Geisinger

Geisinger Health Plan Policies and Procedure Manual

Policy: MPA G2159 – β - Hemolytic Streptococcus Testing

Section: Medical Policy

Subject: β - Hemolytic Streptococcus Testing

Applicable line of business:

Commercial	**x**	**Medicaid**	**x**
Medicare	**x**	**ACA**	**x**
CHIP	**x**		

I. Policy: β - Hemolytic Streptococcus Testing

II. Purpose/Objective: To provide a policy of coverage regarding β - Hemolytic Streptococcus Testing

III. Responsibility:

A. Medical Directors
B. Medical Management

IV. Required Definitions

1. Attachment – a supporting document that is developed and maintained by the policy writer or department requiring/authoring the policy.
2. Exhibit – a supporting document developed and maintained in a department other than the department requiring/authoring the policy.
3. Devised – the date the policy was implemented.
4. Revised – the date of every revision to the policy, including typographical and grammatical changes.
5. Reviewed – the date documenting the annual review if the policy has no revisions necessary.

Commercial

Geisinger Health Plan may refer collectively to health care coverage sponsors Geisinger Health Plan, Geisinger Quality Options, Inc., and Geisinger Indemnity Insurance Company, unless otherwise noted. Geisinger Health Plan is part of Geisinger, an integrated health care delivery and coverage organization.

Medicare

Geisinger Gold Medicare Advantage HMO, PPO, and HMO D-SNP plans are offered by Geisinger Health Plan/Geisinger Indemnity Insurance Company, health plans with a Medicare contract. Continued enrollment in Geisinger Gold depends on contract renewal. Geisinger Health Plan/Geisinger Indemnity Insurance Company are part of Geisinger, an integrated health care delivery and coverage organization.

CHIP

Geisinger Health Plan Kids (GHP Kids) is a Children's Health Insurance Program (CHIP) offered by Geisinger Health Plan in conjunction with the Pennsylvania Department of Human Services (DHS). Geisinger Health Plan is part of Geisinger, an integrated health care delivery and coverage organization.

Medicaid

Geisinger Health Plan Family (GHP Family) is a Medical Assistance (Medicaid) insurance program offered by Geisinger Health Plan in conjunction with the Pennsylvania Department of Human Services (DHS). Geisinger Health Plan is part of Geisinger, an integrated health care delivery and coverage organization.

V. Additional Definitions
Medical Necessity or Medically Necessary means Covered Services rendered by a Health Care Provider that the Plan determines are:

a. appropriate for the symptoms and diagnosis or treatment of the Member's condition, illness, disease or injury;
b. provided for the diagnosis, and the direct care and treatment of the Member's condition, illness disease or injury;
c. in accordance with current standards of good medical treatment practiced by the general medical community.
d. not primarily for the convenience of the Member, or the Member's Health Care Provider; and

the most appropriate source or level of service that can safely be provided to the Member. When applied to hospitalization, this further means that the Member requires acute care as an inpatient due to the nature of the services rendered or the Member's condition, and the Member cannot receive safe or adequate care as an outpatient

Medicaid Business Segment
Medically Necessary — A service, item, procedure, or level of care that is necessary for the proper treatment or management of an illness, injury, or disability is one that:

- Will, or is reasonably expected to, prevent the onset of an illness, condition, injury or disability.
- Will, or is reasonably expected to, reduce or ameliorate the physical, mental or developmental effects of an illness, condition, injury or disability.
- Will assist the Member to achieve or maintain maximum functional capacity in performing daily activities, taking into account both the functional capacity of the Member and those functional capacities that are appropriate for Members of the same age.

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, 2024). 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, 2023; Wessels, 2024).

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

Related Policies

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

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

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, (e.g., cough, oral ulcers, 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) For individuals who fail to demonstrate clinical improvement.

b) For individuals who have progressive symptoms or clinical deterioration after the initiation of antibiotic therapy.

c) 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) For individuals with suspected acute rheumatic fever (ARF) or post-streptococcal glomerulonephritis (PSGN), the following testing **MEETS COVERAGE CRITERIA**:

a) Serological titer testing.

b) Anti-streptolysin O immunoassay.

c) Hyaluronidase activity or anti-hyaluronidase immunoassay.

d) Streptokinase activity or anti-streptokinase immunoassay.

5) In cases of suspected viral pharyngitis, bacterial culture testing for streptococci from a throat swab **DOES NOT MEET COVERAGE CRITERIA**.

6) 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 for individuals who have had either a bacterial culture test or a nucleic acid test for a streptococcal infection.

b) As a screening method in an asymptomatic patient.

c) For individuals with suspected viral pharyngitis.

7) For all situations not described above, serological titer testing **DOES NOT MEET COVERAGE CRITERIA**.

8) Simultaneous ordering of **both** direct probe and amplification probe for the same organism in a single encounter **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.

9) For all situations not described above, testing with an anti-streptolysin O immunoassay, a hyaluronidase activity or anti-hyaluronidase immunoassay, **or** a streptokinase activity or anti-streptokinase immunoassay **DOES NOT MEET COVERAGE CRITERIA**.

10) For all situations, 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 (e.g., Solana Strep Complete Assay, Lyra Direct Strep Assay).

b) MALDI-TOF identification of streptococcus.

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

d) Nicotinamide-adenine dinucleotidase activity or anti-nicotinamide-adenine immunoassay.

NOTES:

Note 1: Centor criteria includes tonsillar exudates, tender anterior cervical lymphadenopathy, fever, and absence of cough with each criterion being worth one point (Chow, 2023).

Table of Terminology

Term	Definition
AAOS	American Academy of Orthopaedic Surgeons
AAP	American Association of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
ADB	Anti-DNase B
AHA	American Heart Association
ARF	Acute rheumatic fever
ASK	Anti-streptokinase
ASM	American Society for Microbiology
ASO	Anti-streptolysin O
ATS	American Thoracic Society
C3	Complement component 3
CAP	Community-acquired pneumonia
CDC	Centers for Disease Control and Prevention
CMS	Centers for Medicare and Medicaid Services
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribose nucleic acid
DNases	Deoxyribonucleases
EIA	Enzyme immunoassays
EOS	Early-onset bacterial sepsis
FDA	Food and Drug Administration
GAS	Group A *Streptococcus*
GBS	Group B *Streptococcus*
GCS	Group C *Streptococcus*
GGS	Group G *Streptococcus*
HDA	Helicase-dependent amplification
ICSI	Institute for Clinical Systems Improvement
IDSA	Infectious Diseases Society of America
LDTs	Laboratory developed Tests
LR	Likelihood ratio
MALDI-TOF	Matrix-assisted laser desorption/ionization-Time of flight
NAAT	Nucleic acid amplification test

NADase	Nicotinamide adenine dinucleotidase
NADTs	Rapid antigen detection tests
NICE	National Institute for Health and Care Excellence
OIA	Optical immunoassays
PCR	Polymerase chain reaction
PIDS	Pediatric Infectious Diseases Society
PJI	Prosthetic joint infection
POC	Point of care
PSGN	Post-streptococcal glomerulonephritis
PYR	Pyrrolidonyl aminopeptidase
qPCR	Quantitative PCR
RADT	Rapid antigen diagnostic testing
RIDT	Rapid in vitro diagnostic tests
RNA	Ribonucleic acid
RNATs	Rapid nucleic acid tests
rt-PCR	Real-time polymerase chain reaction
SDSE	*Streptococcus dysgalactiae* subspecies e*quisimilis*
TSA	Trypticase soy agar

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, 2023). "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, 2024). 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 ≥ three can be tested for GAS pharyngitis (Chow, 2023).

Group A *Streptococcus* 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 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, 2024a). 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, 2024b). 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, 2024d). 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, 2024c).

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, 2024).

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, 2024). 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, 2024; 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 individuals, is of special concern since it is implicated in up to 31% of cases of bacteremia without a focus, eight percent of postpartum endometritis, and two percent 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, 2024; Puopolo et al., 2024). "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

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 Tests (NAATs)	NAATs amplify DNA or RNA to detect the presence of microorganisms. Some are 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.	More sensitive than antibody-based testing for streptococcus. Direct NAATs usually require the use 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 are 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) (AACC, 2021);(2) (Gera & McIver, 2013); (3) (Spellerberg & Brandt, 2016); (4) (CDC, 2024a); (5) (Wald, 2024); (6) (Stevens & Bryant, 2024); (7) (Shulman et al., 2012); (8) (Steer & Gibofsky, 2024); (9) (Miller et al., 2018); (10) (Freeman & Roberts, 2023)		

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 Cohen et al. (2016) 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 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. (2016) 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 et al. (2016) 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 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 (2017) evaluated the accuracy of PCR-based testing for GBS in pregnant individuals. 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%. There were 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. (2019) "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. Overall, 68.2% of events were found to occur once, with 29.1% requiring further follow-up. Furthermore, 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).

O. Luiz et al. (2019) 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 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 six months in two children and for as long as one 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” (O. Luiz et al., 2019).

Fraser et al. (2020) 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 (POC) 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. A total of 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).

McCarty et al. (2022) studied the clinical utility on the GenMark Dx ePlex® blood culture identification gram-positive panel. The panel results were evaluated and compared to MALDI-TOF mass spectrometry and traditional antimicrobial susceptibility testing. One hundred Gram-Positive bacteria were represented. “The positive percent agreement (PPA) was 97/97 with 2 false positives.” The study included chart reviews of 80 patients. The average time for organism identification was 24.4 hours faster, and the average time for

optimization was 29.2 hours faster for the eight patients identified with organisms such as streptococci. The authors "confirm high sensitivity and specificity of the FDA-cleared GenMark Dx ePlex BCID-GP Panel compared to MALDI-TOF MS on bacterial isolates and identify opportunities for earlier optimization of antimicrobial therapy that may also be accompanied by potential cost savings (McCarty et al., 2022).

Guidelines and Recommendations

Centers for Disease Control and Prevention

Acute Pharyngitis (CDC, 2024a): Most cases of acute pharyngitis are viral. Only 20-30% of pharyngitis episodes 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. Healthcare providers can use a positive RADT or throat culture as confirmation of group A strep pharyngitis. For children older than three years old healthcare providers should follow up a negative RADT with a throat culture. For all other ages a throat culture after a negative RADT is not routinely indicated (CDC, 2024a).

Scarlet Fever (CDC, 2024b): 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." To confirm scarlet fever with pharyngitis, healthcare providers need to use either a rapid antigen detection test (RADT) or throat culture. 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 children older than three 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, 2024b).

Post-Streptococcal Glomerulonephritis (PSGN) (CDC, 2024c): 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 the throat
- Isolation of group A strep from skin lesions
- Elevated streptococcal antibodies" (CDC, 2024c).

Acute Rheumatic Fever (CDC, 2024d): "The differential diagnosis of acute rheumatic fever is broad due to the various symptoms of the disease. The differential diagnosis may include specific autoimmune diseases, inflammatory diseases, cancers, and other conditions." 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 now includes the addition of subclinical carditis as a major manifestation for low, moderate, and high risk populations" (CDC, 2024d).

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."
- "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).

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, 2024).

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 □-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).

The 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 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 (ACOG)

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). This committee opinion was reaffirmed in 2022.

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

The 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).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

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).

On November 9, 2020, the FDA approved Mesa Biotech, Inc.'s Accula™ Strep A Test, which is a semi-automated, colorimetric polymerase chain reaction (PCR) nucleic acid amplification test "to qualitatively detect Streptococcus pyogenes (Group A βhemolytic Streptococcus, Strep A) bacterial nucleic acid from unprocessed throat swabs that have not undergone prior nucleic acid extraction" (FDA, 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.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
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(ies), 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], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; 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; Streptococcus, group A

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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Revision History

Effective Date	Summary
01/01/2025	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Updated CC6a to include nucleic acid testing, now reads: "a) As a follow-up test for individuals who have had either a bacterial culture test or a nucleic acid test for a streptococcal infection."
12/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency Language that notes "except in these cases" does not match with general formatting of our policies. The "Except in cases of suspected acute rheumatic fever (ARF) or post-streptococcal glomerulonephritis (PSGN)," language that was seen in former CC6 and CC8.c., 8.e., and 8.f. was moved

	into a new CC that addresses tests allowed for ARF and PSGN and a new CC that notes that these tests aren't allowed in other situations. New CC4: "4) For individuals with suspected acute rheumatic fever (ARF) or post-streptococcal glomerulonephritis (PSGN), the following testing MEETS COVERAGE CRITERIA: a) Serological titer testing. b) Anti-streptolysin O immunoassay. c) Hyaluronidase activity or anti-hyaluronidase immunoassay. d) Streptokinase activity or anti-streptokinase immunoassay." And new CC9: "9) For all situations not described above, testing with an anti-streptolysin O immunoassay, a hyaluronidase activity or anti-hyaluronidase immunoassay, or a streptokinase activity or anti-streptokinase immunoassay DOES NOT MEET COVERAGE CRITERIA." Former CC6, now CC7, now reads: "7) For all situations not described above, serological titer testing DOES NOT MEET COVERAGE CRITERIA."
06/01/2022	Initial Policy Implementation

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, 2024). 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 nongenital 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, 2024). 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 human immunodeficiency virus (HIV), and in rare cases, can also result in HSV meningitis or proctitis (Albrecht, 2024). 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, 2024).

This policy is limited to testing for *C. trachomatis*, *N. gonorrhoeae*, *T. pallidum*, *T. vaginalis* (for guidance on *T. vaginalis* in vaginitis, see AHS-M2057-Diagnosis of Vaginitis Including Multi-Target PCR Testing), HSV, and HPV. The following conditions and/or tests are discussed in the corresponding policies:

- Human Immunodeficiency Virus: AHS-M2116
- Hepatitis B and C: AHS-G2036-Hepatitis Testing
- Pediatric Preventive Screening: AHS-G2042
- Cervical Cancer Screening: AHS-G2002
- Pathogen Panel Testing: AHS-G2149

For STI screening in pregnant individuals, please see AHS-G2035-Prenatal Screening (Nongenetic).

Related Policies

Policy Number	Policy Title
AHS-G2002	Cervical Cancer Screening
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2036	Hepatitis Testing
AHS-G2042	Pediatric Preventive Screening
AHS-G2149	Pathogen Panel Testing
AHS-M2057	Diagnosis of Vaginitis
AHS-M2116	Human Immunodeficiency Virus

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

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**.

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 HR-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/tests **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.

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 an individual's illness.

23) Nucleic acid testing to determine antimicrobial susceptibility in *N. gonorrhoeae* or macrolide resistance in *M. genitalium* **DOES NOT MEET COVERAGE CRITERIA**.

24) 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*

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, 2021c).

Note 2: <u>High-risk for Syphilis (Cantor et al., 2016; CDC, 2023a)</u>:

- Sexually active men who have sex with men (MSM)
- Sexually active HIV-positive status
- Having a sexual partner recently diagnosed with a 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: <u>Signs and Symptoms of a Syphilis Infection (CDC, 2018, 2023a)</u>

- 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: <u>High-risk for Chlamydia and/or Gonorrhea (CDC, 2021b, 2024a, 2024d; LeFevre, 2014)</u>:

- 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: <u>Signs and Symptoms of a Chlamydia Infection (CDC, 2021b, 2024a):</u>

- Genital symptoms, including "discharge, burning during urination, unusual sores, or rash"
- Pelvic Inflammatory Disease (PID), 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: <u>Signs and Symptoms of Gonorrhea (CDC, 2024d):</u>

- 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: <u>Signs and Symptoms of Trichomoniasis (CDC, 2023b):</u>

- 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: <u>Signs and Symptoms of *M. genitalium* Infection (CDC, 2021a):</u>

- 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

Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
AGIHO/DGHO	Infectious Diseases Working Party of the German Society for Hematology and Medical Oncology
AIDs	Acquired immune deficiency syndrome
AIN	Anal intraepithelial neoplasia
ASCUS	Atypical squamous cells of undetermined significance
BASHH	British Association for Sexual Health and HIV
BD	Becton Dickinson
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CIA	Chemiluminescence immunoassay
CIN2+	Cervical intraepithelial neoplasia grade 2+
CIN3	Cervical intraepithelial neoplasia grade 3
CLIA	Chemiluminescent assay
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMIA	Chemiluminescence immunoassay
CMS	Centers for Medicare and Medicaid
CNS	Central nervous system
CPS	Canadian Paediatric Society
CSF	Cerebrospinal fluid
CT	*Chlamydia trachomatis*
DAG-KBT	German Working Group for Blood and Marrow Transplantation
DFE	Darkfield examination
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
E7-MPG	E7 multiplex genotyping
EBV	Epstein Barr virus
ED	Emergency department
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FEMS	Federation of European Microbiological Societies
FIA	Fluorescence immunoassay
FNA	Fine needle aspiration
FTA	Fluorescent treponemal antibody
GC	Gonococcal
gG2	Glycoprotein G2

GP5+/6+	General primer 5+/6+
HBV	Hepatitis B
HC2	Hybrid capture 2
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus-1
HPV	Human papillomavirus
HPV-16	Human papillomavirus type 16
HPV-18	Human papillomavirus type 18
HR-HPV	High risk or oncogenic HPV testing
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus-1
HSV-2	Herpes simplex virus-2
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IMCA	Immunochemiluminometric assay
ISH	In situ hybridization
ISVVD	The International Society for the Study of Vulvovaginal Disease
IUSTI	International Union Against Sexually Transmitted Infections
JAMA	Journal of the American Medical Association
LDTs	Laboratory-Developed Tests
LGSIL	Low grade squamous intraepithelial lesion on cytologic smear of anus
LGV	Lymphogranuloma venereum
LSIL	Low-grade squamous intraepithelial lesions
MG	*Mycoplasma genitalium*
Mgen	*Mycoplasma genitalium*
MHA-TP	Microhemagglutination Assay for *Treponema pallidum* antibodies
MLST	Multilocus sequence typing
mRNA	Messenger RNA
MSM	Men having sex with men
MTC	Male Training Center for Family Planning & Reproductive Health
NA	Not applicable
NAAT	Nucleic acid amplification testing
NCCN	National Comprehensive Cancer Network
NG	Neisseria gonorrhoeae
NGU	Nongonococcal urethritis
NICE	National Institute for Health and Care Excellence
NOS	Not otherwise specified
NTT	Nontreponemal test
ORPH-1	Oropharynx-1
OS	Overall survival
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
POC	Point-of-care
POCT	Point-of-care test
PrEP	Preexposure prophylaxis
PWID	People who inject drugs
RFLP	Restriction fragment length polymorphism

RNA	Ribonucleic acid
RPR	Rapid plasma reagin test
SDA	Strand displacement amplification
STDs	Sexually transmitted diseases
STIs	Sexually transmitted infections
TMA	Transcription-mediated amplification
TOC	Test of cure
TPHA	*Treponema pallidum* hemagglutination
TP-IgA	*Treponema pallidum* IgA antibodies
TPPA	*Treponema pallidum* particle agglutination
TP-PA	*T. pallidum* passive particle agglutination
TT	Treponemal test
TV	Trichomonas vaginalis
USPSTF	United States Preventive Services Task Force
VDRL	Venereal disease research laboratory
VIN	Vulvar intraepithelial neoplasia

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, 2024). In the U.S. alone, in 2022, over 1.6 million cases of chlamydia were reported to the CDC, but the CDC estimates that 2.86 million chlamydial infections occur annually (CDC, 2024a, 2024h). 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, 2024a).

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, 2021a).

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, 2024). 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 2022, the CDC reported an 11% increase since 2018 in the number of cases of gonorrhea reported in the United States (CDC, 2024g). The CDC also reported 207,255 new cases of gonorrhea in the United States in 2018 (CDC, 2024g).

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 2021, 2855 cases of congenital syphilis were reported. This included 220 syphilis-related stillbirths and infant deaths (Hicks & Clement, 2023).

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, 2023).

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, 2022). 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). In 2018, CDC estimates show there were 572,000 new genital herpes infections in the U.S. among people aged 14 to 49; 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, 2024b). 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, 2024). 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, 2024).

Human Papillomavirus (HPV)

Anogenital HPV infection is the most common STI worldwide with an estimation that "almost all sexually active individuals will acquire HPV at some point in their lifetime" (Palefsky, 2024). 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 been approved for use in the U.S., and the CDC recommends vaccination for HPV for all children ages 11 or 12 (CDC, 2024c). 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, 2024).

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 acquired immune deficiency syndrome (AIDs). In the 2019 issue of JAMA, the U.S. 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, 2022; 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).

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, 2022). 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, 2022).

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, 2022):

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, 2022). 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, 2022). Sensitivity and specificity of this test are dependent on the manner in which samples were collected, with patient collected vaginal swab, endocervical swab, urine, and pharyngeal swab specimens showing sensitivity and specificity in the mid to high ninetieth percentile. Rectal swab specimens showed a lower sensitivity for both CT (86%) and NG (91.2%), but

specificity in the 99th percentile, similar to the specificity of the other sample collection methods (Cepheid, 2022).

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 (Golden et al.), 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 U.S. Preventive Services Task Force (USPSTF) conducted a systematic review of the use of serologic screening for genital herpes and published their findings in 2016 (USPSTF, 2023). 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" (USPSTF, 2023).

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 (Golden et al.) 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 point-of-care test (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 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 (USPSTF, 2023) 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.

Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

Anal Carcinoma (NCCN, 2023a): 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 (Marcell & Health) "can be identified by cytology, HPV testing, digital rectal examination, 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.

Cervical Cancer (NCCN, 2024a): "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 child-bearing.

Head and Neck Cancers (NCCN, 2024b): 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 correlates with HPV status in geographic regions where HPV is etiologically responsible for a high proportion of cancers. Confirmatory HPV direct testing is recommended, especially for clinical trials. Clinical centers are recommended to ascertain concordance rate of p16 and direct HPV testing, as this may vary by region, if considering use of p16 IHC alone as a surrogate.

- Distinguishing p16+ patients by HPV tumor status informs prognosis. Patients with p16+ and HPV+ tumors have an improved prognosis compared to patients with p16+ and HPV-negative tumors.
- Direct HPV confirmatory tests include polymerase chain reaction (PCR) and RNA in situ hybridization (ISH).
- PCR may provide additional sensitivity while ISH provides increased specificity.
- 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.
- When using p16, the 70% cutoff with nuclear and cytoplasmic expression with at least moderate to strong intensity is recommended."

Occult Primary Cancers (NCCN, 2024d): 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 p16 immunohistochemistry/human papillomavirus (HPV) in situ hybridization (ISH) and Epstein-Barr virus (EBV) (ISH); 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, 2024d).

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, 2024c): "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, cervical HPV testing, and cytology testing." The guidelines also note for the surveillance of vulvar cancer: "Annual cervical/vaginal cytology tests, which may include HPV testing, can be considered as indicated for detection of lower genital tract dysplasia, although its value in detecting recurrent cancers is limited and the likelihood of detecting asymptomatic recurrence is low." (NCCN, 2024c).

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 (USPSTF, 2023): 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 infection in asymptomatic adolescents and adults, including pregnant persons."

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, 2021b). 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, 2024b).

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, 2021b).

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, 2021b).

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, 2024f). 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, 2021b).

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, 2021b).

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, 2021b). 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.

Gonorrhea has developed resistance to nearly all the antibiotics used for its treatment, creating a need for research into identifying genetic mutations in the pathogen that are contributing to the antibiotic resistance. However, according to the CDC "currently, there is no well-studied, reliable technology that allows for antibiotic susceptibility testing from nonculture specimens. Increased laboratory culture capacity is needed" (CDC, 2024e). CDC recommends that all state and local health department labs maintain or develop the capacity to perform gonorrhea culture, or form partnerships with experienced laboratories that can perform this type of testing.

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, 2021b). 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, 2021a).

"*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, 2021a). The CDC then goes on to add, if available, men with recurrent NGU should be tested for *M. genitalium* using an FDA-cleared NAAT, resistance testing 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. Testing should be accompanied with resistance testing (CDC, 2021a).

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, 2021b).

The CDC (2024c) 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 (2019) 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);
- 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 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 (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. 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).

Canadian Guidelines on Sexually Transmitted Infections

"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 (Marcell & Health) 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" (NICE, 2018).

Canadian Paediatric Society (CPS)

The 2024 update to the CPS practice point titled “Diagnosis and management of congenital syphilis – Avoiding missed opportunities" included the following:

> “The potential for asymptomatic syphilis infection and its nonspecific or subtle maternal disease manifestations make serology the cornerstone of diagnosis. At a minimum, syphilis serology is recommended at the time of the first prenatal visit, with recommendations for repeat testing at 28 to 32 weeks and at delivery in areas with outbreaks or for individuals with ongoing risk of infection. Repeat testing should also be performed in the context of clinical suspicion of maternal reinfection, a new maternal STI at any point during pregnancy (e.g., gonorrhea, chlamydia), in case of a stillbirth after 20 weeks gestation, or in accordance with provincial/territorial guidelines. Newborn infants ideally should not be discharged from hospital until results of maternal syphilis testing are known and appropriate steps for management are arranged. ” (Society, 2024).

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 (e.g., 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 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	Follow-up testing depends on the nature of infection, as follows: 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	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

	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	– 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 et al., 2016).

Cumulative Guideline Table

Year & Society	Condition	Microorganism	Recommendation
2023 NCCN	Anal Carcinoma	HPV	HPV linked to anal cancers and HPV positivity linked to positive OS
2024 NCCN	Cervical Cancer	HPV	Overwhelming evidence of link between HPV and cervical cancer; chronic HPV infection status used in aiding treatment/surgical options
2024 NCCN	Head and Neck Cancers/ Oropharyngeal Cancer	HPV	Requires HPV p16 testing by IHC; HPV status is imperative in determining therapy
2024 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
2023 NCCN	Penile Cancer	HPV	HPV linked to penile cancer; HPV status of lesions important for determining therapy
2024 NCCN	Vulvar Cancer (Squamous Cell Carcinoma)	HPV	HPV linked to vulvar cancer, especially HSIL; recommends HPV testing for "appropriate patients"

Year & Society	Condition	Microorganism	Recommendation
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
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.

Year & Society	Condition	Microorganism	Recommendation
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."
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
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

Year & Society	Condition	Microorganism	Recommendation
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.
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.

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			

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

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.

Applicable CPT/HCPCS Procedure Codes

CPT	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 (eg, VDRL, RPR, ART)
86593	Syphilis test, non-treponemal antibody; quantitative
86631	Antibody; Chlamydia
86632	Antibody; Chlamydia, IGM
86694	Antibody; herpes simplex, non-specific type test
86695	Antibody; herpes simplex, type 1

CPT	Code Description
86696	Antibody; herpes simplex, type 2
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)
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
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (eg, 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
87563	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma genitalium, amplified probe technique
87590	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, direct 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 (eg, 6, 11, 42, 43, 44)
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (eg, 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
87660	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique

CPT	Code Description
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 (ie, visual) 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
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure
0064U	Antibody, Treponema pallidum, total and rapid plasma reagin (RPR), immunoassay, qualitative Proprietary test: BioPlex 2200 Syphilis Total & RPR Assay Lab/Manufacturer: Bio-Rad Laboratories
0065U	Syphilis test, non-treponemal antibody, immunoassay, qualitative (RPR) Proprietary test: BioPlex 2200 RPR Assay Lab/Manufacturer: Bio-Rad Laboratories
0096U	Human papillomavirus (HPV), high-risk types (ie, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), male urine Proprietary test: HPV, High-Risk, Male Urine Lab/Manufacturer: Molecular Testing Labs/Roche Cobas
0210U	Syphilis test, non-treponemal antibody, immunoassay, quantitative (RPR) Proprietary test: BioPlex 2200 RPR Assay - Quantitative Lab/Manufacturer: Bio-Rad Laboratories
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
0455U	Infectious agents (sexually transmitted infection), Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis, multiplex amplified probe technique, vaginal, endocervical, gynecological specimens, oropharyngeal swabs, rectal swabs, female or male urine, each pathogen reported as detected or not detected Proprietary test: Abbott AlinityTM m STI Assay Lab/Manufacturer: Abbott Molecular, Inc
0463U	Oncology (cervix), mRNA gene expression profiling of 14 biomarkers (E6 and E7 of the highest-risk human papillomavirus [HPV] types 16, 18, 31, 33, 45, 52, 58), by real-time nucleic acid sequence-based amplification (NASBA), exo- or endocervical

CPT	Code Description
	epithelial cells, algorithm reported as positive or negative for increased risk of cervical dysplasia or cancer for each biomarker Proprietary test: Proofer '7 HPV mRNA E6 and E7 Biomarker Test Lab/Manufacturer: Global Diagnostics Labs, LLC, PreTect AS, a Mel-Mont Medical, Inc
0483U	Infectious disease (Neisseria gonorrhoeae), sensitivity, ciprofloxacin resistance (gyrA S91F point mutation), oral, rectal, or vaginal swab, algorithm reported as probability of fluoroquinolone resistance Proprietary test: Ciprofloxacin Susceptibility of Neisseria gonorrhoeae Lab/Manufacturer: MedArbor Diagnostics, SpeeDx, Inc
0484U	Infectious disease (Mycoplasma genitalium), macrolide sensitivity (23S rRNA point mutation), oral, rectal, or vaginal swab, algorithm reported as probability of macrolide resistance Proprietary test: Macrolide Resistance of Mycoplasma genitalium Lab/Manufacturer: MedArbor Diagnostics, SpeeDx, Inc
0500T	Infectious agent detection by nucleic acid (DNA or RNA), Human Papillomavirus (HPV) for five or more separately reported high-risk HPV types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) (ie, genotyping)
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

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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Revision History

Effective Date	Summary
01/01/2025	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: New CC23: "23) Nucleic acid testing to determine antimicrobial susceptibility in N. gonorrhoeae or macrolide resistance in M. genitalium DOES NOT MEET COVERAGE CRITERIA." Added CPT code 0483U, 0484U Removed CPT code 0167U (deleted code; effective date 10/1/2024) Off-cycle coding modification: Added CPT code 0455U, 0463U (effective date 07/01/2024) Removed CPT code 0353U (effective date 07/01/2024) Off-cycle coding modification: Removed CPT code 0354U (effective date 04/01/2024)
12/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: Addition of CC 1d: "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)." Addition of bullet points to Note 3, signs and symptoms of neuro, ocular, and otosyphilis and late/tertiary syphilis: "• 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)." Moved Trichomonas CC from being CC17/18 to being CC10/11. Addition of new CC12 and CC13: "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." Former CC10, now CC14, edited to expand panel coverage from chlamydia and gonorrhea to those plus trichomonas and M.genitalium. CC now reads: "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." Added CPT code 87563, 0402U (effective date 10/1/2023)
06/01/2022	Initial Policy Implementation

EXCLUSIONS:
Note: A complete description of the process by which a given technology or service is evaluated and determined to be experimental, investigational or unproven is outlined in MP 15 - Experimental Investigational or Unproven Services or Treatment.

Medicaid Business Segment:

Any requests for services, that do not meet criteria set in the PARP, may be evaluated on a case by case basis.

LINE OF BUSINESS:
Eligibility and contract specific benefits, limitations and/or exclusions will apply. Coverage statements found in the line of business specific benefit document will supersede this policy. For Medicare, applicable LCD's and NCD's will supercede this policy. For PA Medicaid Business segment, this policy applies as written.

Geisinger Health Plan may refer collectively to health care coverage sponsors Geisinger Health Plan, Geisinger Quality Options, Inc., and Geisinger Indemnity Insurance Company, unless otherwise noted. Geisinger Health Plan is part of Geisinger, an integrated health care delivery and coverage organization.

Coverage for experimental or investigational treatments, services and procedures is specifically excluded under the member's certificate with Geisinger Health Plan. Unproven services outside of an approved clinical trial are also specifically excluded under the member's certificate with Geisinger Health Plan. This policy does not expand coverage to services or items specifically excluded from coverage in the member's certificate with Geisinger Health Plan. Additional information can be found in MP015 Experimental, Investigational or Unproven Services.

Prior authorization and/or pre-certification requirements for services or items may apply. Pre-certification lists may be found in the member's contract specific benefit document. Prior authorization requirements can be found at https://www.geisinger.org/health-plan/providers/ghp-clinical-policies