

## Diagnostic Testing of Common Sexually Transmitted Infections

Policy Number: AHS – G2157 – Diagnostic Testing of Common Sexually Transmitted Infections	Prior Policy Name and Number, as applicable:  Portions of this policy replaces portions of M2097- Identification of Microorganisms using Nucleic Acid Probes
Initial Presentation Date: 09/25/2018 Revision Date: 09/04/2024	

[POLICY DESCRIPTION](#) | [RELATED POLICIES](#) | [INDICATIONS AND/OR LIMITATIONS OF COVERAGE](#) | [TABLE OF TERMINOLOGY](#) | [SCIENTIFIC BACKGROUND](#) | [GUIDELINES AND RECOMMENDATIONS](#) | [APPLICABLE STATE AND FEDERAL REGULATIONS](#) | [APPLICABLE CPT/HCPCS PROCEDURE CODES](#) | [EVIDENCE-BASED SCIENTIFIC REFERENCES](#) | [REVISION HISTORY](#)

### I. Policy Description

Sexually transmitted infections (STIs), often referred to as sexually transmitted diseases or STDs, include a variety of pathogenic bacteria, virus, and other microorganisms that are spread through sexual contact and can cause a multitude of complications if left untreated. Chlamydia and gonorrhea, caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, respectively, have high rates of occurrence in the United States and can cause pelvic inflammatory disease (PID), infertility, and pregnancy complications. The causative agent of syphilis is *Treponema pallidum*; if left untreated, syphilis can lead to serious cardiac and neurological conditions (Ghanem & Tuddenham, 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).

## II. 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

## III. 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.

*This document is subject to the disclaimer found at <https://www.medmutual.com/LabPolicies>. If printed, this document is subject to change. Always verify the most current version of the official document at <https://www.medmutual.com/LabPolicies>.*

- 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: High-risk for Syphilis (Cantor et al., 2016; CDC, 2023a):**

- 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: Signs and Symptoms of a Syphilis Infection (CDC, 2018, 2023a)**

- Chancre
- Skin rash and/or mucous membrane lesions in mouth, vagina, anus, hands, and feet

This document is subject to the disclaimer found at <https://www.medmutual.com/LabPolicies>. If printed, this document is subject to change. Always verify the most current version of the official document at <https://www.medmutual.com/LabPolicies>.



- Condyloma lata
- Secondary symptomology can include fever, fatigue, sore throat, swollen lymph nodes, weight loss, muscle aches, headache, and hair loss
- Signs and symptoms of neurosyphilis can include severe headache, trouble with muscle movements, muscle weakness or paralysis (not being able to move certain parts of the body), numbness, and changes in mental status (trouble focusing, confusion, personality change) and/or dementia (problems with memory, thinking, and/or making decisions).
- Signs and symptoms of ocular syphilis can include eye pain or redness, floating spots in the field of vision (“floaters”), sensitivity to light, and changes in vision (blurry vision or even blindness).
- Signs and symptoms of otosyphilis may include hearing loss, ringing, buzzing, roaring, or hissing in the ears (“tinnitus”), balance difficulties, and dizziness or vertigo.
- Signs and symptoms of late/tertiary syphilis include inflammatory lesions of the cardiovascular system (e.g., aortitis, coronary vessel disease), skin (e.g., gummatous lesions), and bone (e.g., osteitis).

**Note 4: High-risk for Chlamydia and/or Gonorrhea (CDC, 2021b, 2024a, 2024d; LeFevre, 2014):**

- Sexually active men who have sex with men (MSM)
- Sexually active HIV-positive status
- Sexually active women under the age of 25
- Women age 25 or over who have multiple sexual partners
- Having a sexual partner recently diagnosed with an STI
- Previous or concurrent STI
- Exchanging sex for money or drugs

**Note 5: Signs and Symptoms of a Chlamydia Infection (CDC, 2021b, 2024a):**

- 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: Signs and Symptoms of Gonorrhea (CDC, 2024d):**

- Dysuria
- Urethral infection

- Urethral or vaginal discharge
- Epididymitis (Testicular or scrotal pain)
- Rectal infection symptoms include anal itching, discharge, rectal bleeding, and painful bowel movements

**Note 7: Signs and Symptoms of Trichomoniasis (CDC, 2023b):**

- Vaginal or penile discharge
- Itching, burning sensation, or soreness of the genitalia
- Discomfort or burning sensation during/after urination and/or ejaculation
- Urethritis
- Epididymitis
- Prostatitis

**Note 8: Signs and Symptoms of *M. genitalium* Infection (CDC, 2021a):**

- 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

#### IV. 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	<i>Chlamydia trachomatis</i>
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	<i>Mycoplasma genitalium</i>

Mgen	<i>Mycoplasma genitalium</i>
MHA-TP	Microhemagglutination Assay for <i>Treponema pallidum</i> 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	<i>Neisseria gonorrhoeae</i>
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	<i>Treponema pallidum</i> hemagglutination
TP-IgA	<i>Treponema pallidum</i> IgA antibodies
TPPA	<i>Treponema pallidum</i> particle agglutination
TP-PA	<i>T. pallidum</i> passive particle agglutination
TT	Treponemal test
TV	<i>Trichomonas vaginalis</i>
USPSTF	United States Preventive Services Task Force
VDRL	Venereal disease research laboratory
VIN	Vulvar intraepithelial neoplasia

## V. Scientific Background

### *Chlamydia*

This document is subject to the disclaimer found at <https://www.medmutual.com/LabPolicies>. If printed, this document is subject to change. Always verify the most current version of the official document at <https://www.medmutual.com/LabPolicies>.

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 and 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):

<b>Provide the following services:</b>	<b>Screening tests/samples</b>
<b>At 3 months after PrEP initiation:</b>	<ul style="list-style-type: none"> <li>• Test for HIV.</li> <li>• Measure serum creatinine and estimate creatinine clearance.</li> <li>• Provide medication adherence and behavioral risk reduction support.</li> <li>• Additionally, for               <ul style="list-style-type: none"> <li>o MSM: screen for bacterial STIs*;</li> <li>o Women with reproductive potential: test for pregnancy; and</li> <li>o PWID: assess access to sterile needles/syringes and to drug treatment services.</li> </ul> </li> </ul>
<b>Every 3 months after the first 3-month follow-up</b>	<ul style="list-style-type: none"> <li>• Test for HIV.</li> <li>• Provide medication adherence and behavioral risk reduction support.</li> <li>• Additionally, for               <ul style="list-style-type: none"> <li>o MSM: screen for bacterial STIs*;</li> <li>o Women with reproductive potential: test for pregnancy; and</li> <li>o PWID: assess access to sterile needles/syringes and to substance use disorder treatment services.</li> </ul> </li> </ul>
<b>Every 6 months after the first 3-month follow-up</b>	<ul style="list-style-type: none"> <li>• Measure serum creatinine and estimate creatinine clearance.</li> <li>• For all sexually active patients: Screen for bacterial STIs*.</li> </ul>

\*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 99<sup>th</sup> 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.

## VI. 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  $\geq 10$  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 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 bacteraemia, 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.<sup>69</sup> 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 to detect sexually transmitted infections?

What screening tests should be used to detect sexually transmitted infections?		
Infection	Screening tests/samples	Follow-up testing
<b>Chlamydia</b>	<p>NAAT is the most sensitive and specific test. Can be performed on urine, urethral swabs, vaginal or cervical swabs*</p> <p>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.</p> <p>For pharyngeal and rectal specimens, NAAT may be considered; discuss with testing laboratory</p>	<p>Test-of-cure 3 to 4 weeks after treatment:</p> <ul style="list-style-type: none"> <li>– Compliance is uncertain</li> <li>– Second-line or alternative treatment was used</li> <li>– Re-exposure risk is high</li> <li>– An adolescent is pregnant</li> </ul>
<b>Syphilis</b>	<p>Serology remains the usual diagnostic test unless the patient has lesions compatible with syphilis</p> <p>Treponemal-specific screening assays (e.g., EIA) are more sensitive than non-treponemal tests, though testing algorithms vary across jurisdictions</p> <p>If treponemal-specific assay is positive, a second treponemal test is usually required</p>	<p>Follow-up testing depends on the nature of infection, as follows:</p> <p>Primary, secondary, early latent infection: Repeat serology at 1, 3, 6, and 12 months after treatment</p> <p>Late latent infection: Repeat serology 12 and 24 months after treatment</p> <p>Neurosyphilis: Repeat 6, 12, and 24 months after treatment</p>
<b>Gonorrhea</b>	<p>NAAT can be used to detect gonorrhea from urine, and urethral, vaginal and cervical swabs in symptomatic and asymptomatic individuals*</p> <p>Culture allows for antimicrobial susceptibility testing and should be performed if a patient does not promptly respond to therapy</p> <p>Cultures should be submitted for asymptomatic or symptomatic MSM, who have an increased incidence of antibiotic resistance</p> <p>For rectal and pharyngeal testing, discuss preferred specimens with the testing</p>	<p>Test-of-cure (culture 3 to 7 days post-treatment or NAAT 2 to 3 weeks later) if:</p> <ul style="list-style-type: none"> <li>– Second-line or alternative treatment was used</li> <li>– Antimicrobial resistance is a concern</li> <li>– Compliance is uncertain</li> <li>– Re-exposure risk is high</li> <li>– An adolescent is pregnant</li> <li>– Previous treatment failure</li> <li>– Pharyngeal or rectal infection</li> <li>– Infection is disseminated</li> </ul>

	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	– 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  $\leq 12$  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**

<b>Year &amp; Society</b>	<b>Condition</b>	<b>Microorganism</b>	<b>Recommendation</b>
<b>2023 NCCN</b>	Anal Carcinoma	HPV	HPV linked to anal cancers and HPV positivity linked to positive OS
<b>2024 NCCN</b>	Cervical Cancer	HPV	Overwhelming evidence of link between HPV and cervical cancer; chronic HPV infection status used in aiding treatment/surgical options
<b>2024 NCCN</b>	Head and Neck Cancers/ Oropharyngeal Cancer	HPV	Requires HPV p16 testing by IHC; HPV status is imperative in determining therapy
<b>2024 NCCN</b>	Occult Primary Cancers (Squamous Cell Carcinoma)	HPV	If clinical presentation in the head and neck nodes is noted, check p16 IHC and ISH results

<b>Year &amp; Society</b>	<b>Condition</b>	<b>Microorganism</b>	<b>Recommendation</b>
<b>2023 NCCN</b>	Penile Cancer	HPV	HPV linked to penile cancer; HPV status of lesions important for determining therapy
<b>2024 NCCN</b>	Vulvar Cancer (Squamous Cell Carcinoma)	HPV	HPV linked to vulvar cancer, especially HSIL; recommends HPV testing for “appropriate patients”
<b>2021 USPSTF</b>	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
<b>2014 USPSTF</b>	Oropharyngeal Cancer	HPV	Insufficient evidence to assess testing for HPV in cases of asymptomatic oropharyngeal cancer
<b>2016 USPSTF</b>	Asymptomatic Genital Herpes	HSV-2	Do not recommend testing asymptomatic patients for HSV-2
<b>2016 USPSTF</b>	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
<b>2021 CDC</b>	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
<b>2021 CDC</b>	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
<b>2021 CDC</b>	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

Year & Society	Condition	Microorganism	Recommendation
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	<p>Recommends against using oncogenic HPV testing for asymptomatic men, women aged 25 and over, or for general STI testing.</p> <p>There is no approved test for HPV in men, and routine testing is not recommended for anal, penile, or throat cancers in men.</p>
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	<p>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</p> <p>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.</p>
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.



Year & Society	Condition	Microorganism	Recommendation
<b>2019 IUSTI</b>	Lymphogranuloma venereum	Chlamydia	To diagnose LGV, a sample tested <i>C. trachomatis</i> 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.
<b>2014, 2020 IUSTI</b>	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
<b>2015 IUSTI (published in 2016)</b>	NA	Chlamydia	Recommends using an NAAT for chlamydia testing and lists signs/symptoms that require testing
<b>2017 IUSTI</b>	Genital herpes	HSV	Typically, does not recommend testing in asymptomatic patients; HSV DNA detection now replaces culture as gold standard
<b>2014 MTC</b>	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
<b>2014 MTC</b>	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
<b>2014 MTC</b>	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)
<b>2017 Canadian Guidelines on STIs</b>	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.

Year & Society	Condition	Microorganism	Recommendation
<b>2014 AAP</b>	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.
<b>2014 AAP</b>	Adolescents & young adults	Syphilis	Do NOT recommend routine screening except for sexually active young MSM.
<b>2016 NICE</b>	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
<b>2018 CPS</b>	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
<b>2020 CPS</b>	Adolescents/young adults	Chlamydia, Syphilis, Gonorrhea	See detailed testing and frequency in table within the guidelines above
<b>2015 BASHH (published in 2016)</b>	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.
<b>2013 BASHH</b>	Suspected LGV	Chlamydia	Testing should use either NAAT, culture testing, or serology; however, the latter cannot distinguish current from past infections.
<b>2014 BASHH (published in 2015)</b>	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).
<b>2015, 2018 BASHH</b>	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.

Year & Society	Condition	Microorganism	Recommendation
<b>Abbreviations:</b> 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			

## VII. 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.

## VIII. 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
86696	Antibody; herpes simplex, type 2
86701	Antibody; HIV-1
86702	Antibody; HIV-2

CPT	Code Description
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); <i>Trichomonas vaginalis</i> , 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; <i>Trichomonas vaginalis</i>
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, <i>Treponema pallidum</i> , 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), <i>Chlamydia trachomatis</i> , <i>Neisseria gonorrhoeae</i> , <i>Trichomonas vaginalis</i> , <i>Mycoplasma genitalium</i> , 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), <i>Chlamydia trachomatis</i> , <i>Neisseria gonorrhoeae</i> , and <i>Trichomonas vaginalis</i> , 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 Alinity™ m STI Assay Lab/Manufacturer: Abbott Molecular, Inc



CPT	Code Description
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 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

Current Procedural Terminology© American Medical Association. All Rights reserved.  
*Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.*

## IX. Evidence-based Scientific References

Abbott. (2023). *Alinity m STI AMP Kit*. <https://www.molecularcatalog.abbott/int/en/alinity-m-sti-assay>

- Albrecht, M. A. (2024, June 20). *Epidemiology, clinical manifestations, and diagnosis of genital herpes simplex virus infection*. <https://www.uptodate.com/contents/epidemiology-clinical-manifestations-and-diagnosis-of-genital-herpes-simplex-virus-infection>
- Allen, U. D., MacDonald, N. E., & Top, K. (2019). *Diagnosis and management of sexually transmitted infections in adolescents*. <https://www.cps.ca/en/documents/position/sexually-transmitted-infections>
- Arbyn, M., Roelens, J., Simoons, C., Buntinx, F., Paraskevaidis, E., Martin-Hirsch, P. P., & Prendiville, W. J. (2013). Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. *Cochrane Database Syst Rev*(3), Cd008054. <https://doi.org/10.1002/14651858.CD008054.pub2>
- BASHH. (2018, 09/26/2018). *BASHH CLINICAL EFFECTIVENESS GROUP Update on the treatment of Chlamydia trachomatis (CT) infection*. <https://www.bashhguidelines.org/current-guidelines/urethritis-and-cervicitis/chlamydia-2015/>
- BD. (2020). *BD receives FDA Approval for HPV Test with Extended Genotyping Capabilities*. <https://www.bd.com/en-us/company/news-and-media/press-releases/july-22-2020-bd-receives-fda-approval-for-hpv-test-with-extended-genotyping-capabilities>
- Brischetto, A., Gassiep, I., Whiley, D., & Norton, R. (2018). Retrospective Review of Treponema pallidum PCR and Serology Results: Are Both Tests Necessary? *J Clin Microbiol*, 56(5). <https://doi.org/10.1128/jcm.01782-17>
- Bristow, C. C., Morris, S. R., Little, S. J., Mehta, S. R., & Klausner, J. D. (2019). Meta-analysis of the Cepheid Xpert® CT/NG assay for extragenital detection of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections. *Sex Health*, 16(4), 314-319. <https://doi.org/10.1071/sh18079>
- Cantor, A. G., Pappas, M., Daeges, M., & Nelson, H. D. (2016). Screening for syphilis: Updated evidence report and systematic review for the us preventive services task force. *JAMA*, 315(21), 2328-2337. <https://doi.org/10.1001/jama.2016.4114>
- Castle, P. E., Stoler, M. H., Wright, T. C., Jr., Sharma, A., Wright, T. L., & Behrens, C. M. (2011). Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *Lancet Oncol*, 12(9), 880-890. [https://doi.org/10.1016/s1470-2045\(11\)70188-7](https://doi.org/10.1016/s1470-2045(11)70188-7)
- CDC. (2018, 04/16/2021). *Syphilis (Treponema pallidum): 2018 Case Definition*. <https://ndc.services.cdc.gov/case-definitions/syphilis-2018/>
- CDC. (2019, 04/18/2022). *HPV & Men Fact Sheet*. <https://npin.cdc.gov/publication/hpv-and-men-cdc-fact-sheet-0>
- CDC. (2021a, 07/22/2021). *Mycoplasma genitalium*. <https://www.cdc.gov/std/treatment-guidelines/mycoplasmagenitalium.htm>
- CDC. (2021b). *Sexually Transmitted Infections Treatment Guidelines, 2021*. Retrieved 07/28/2021 from <https://www.cdc.gov/std/treatment-guidelines/STI-Guidelines-2021.pdf>
- CDC. (2021c). *Sexually Transmitted Infections Treatment Guidelines, 2021 - Adolescents*. <https://www.cdc.gov/std/treatment-guidelines/adolescents.htm>
- CDC. (2022). *Pre-Exposure Prophylaxis (PrEP)*. Centers for Disease Control and Prevention. <https://www.cdc.gov/hiv/risk/prep/index.html>
- CDC. (2023a, 12/08/2023). *About Syphilis*. <https://www.cdc.gov/syphilis/about/index.html>
- CDC. (2023b, 12/13/2023). *About Trichomoniasis*. <https://www.cdc.gov/trichomoniasis/about/index.html>

- CDC. (2024a, 02/20/2024). *About Chlamydia*. <https://www.cdc.gov/chlamydia/about/index.html>
- CDC. (2024b, 02/20/2024). *About Genital Herpes*. <https://www.cdc.gov/herpes/about/index.html>
- CDC. (2024c, 02/06/2024). *About Genital HPV Infection*. <https://www.cdc.gov/sti/about/about-genital-hpv-infection.html>
- CDC. (2024d, 02/15/2024). *About Gonorrhea*. <https://www.cdc.gov/gonorrhea/about/index.html>
- CDC. (2024e, February 15). *Drug-Resistant Gonorrhea*. <https://www.cdc.gov/gonorrhea/hcp/drug-resistant/index.html>
- CDC. (2024f, March 7). *Neurosyphilis, Ocular Syphilis, and Ootosyphilis*. <https://www.cdc.gov/syphilis/hcp/neurosyphilis-ocular-syphilis-otosyphilis/>
- CDC. (2024g, April 11). *The State of STIs - Infographic*. Centers for Disease Control and Prevention. <https://www.cdc.gov/sti/media/pdfs/TheStateOfSTIs.pdf>
- CDC. (2024h, January 30, 2024). *Table 2. Chlamydia — Reported Cases and Rates of Reported Cases by State, Ranked by Rates, United States, 2022*. <https://www.cdc.gov/std/statistics/2022/tables/2.htm>
- Cepheid. (2022). *Xpert® CT/NG*. <https://www.cepheid.com/Package%20Insert%20Files/Xpert-CTNG-US-ENGLISH-Package-Insert-301-0234--Rev-K.pdf>
- Chernesky, M., Fisher, W. A., Gale-Rowe, M., Labbé, A., Lau, T. T. Y., Lee, E., Martin, I., Ogilvie, G., Read, R., Robinson, J., Romanowski, B., Ryan, B., Singh, A., Steben, M., Wong, T., & Yudin, M. H. (2017, 04/20/2017). *Canadian Guidelines on Sexually Transmitted Infections-Laboratory diagnosis of sexually transmitted infections*. Public Health Agency of Canada. [https://ipac-canada.org/photos/custom/Members/pdf/Laboratory%20Diagnosis%20of%20STI\\_April%202017\\_final-5.pdf](https://ipac-canada.org/photos/custom/Members/pdf/Laboratory%20Diagnosis%20of%20STI_April%202017_final-5.pdf)
- Cook, R. L., Hutchison, S. L., Ostergaard, L., Braithwaite, R. S., & Ness, R. B. (2005). Systematic review: noninvasive testing for Chlamydia trachomatis and Neisseria gonorrhoeae. *Ann Intern Med*, 142(11), 914-925.
- Cosentino, L. A., Danby, C. S., Rabe, L. K., Macio, I., Meyn, L. A., Wiesenfeld, H. C., & Hillier, S. L. (2017). Use of Nucleic Acid Amplification Testing for Diagnosis of Extragenital Sexually Transmitted Infections. *J Clin Microbiol*, 55(9), 2801-2807. <https://doi.org/10.1128/jcm.00616-17>
- Davidson, K. W., Barry, M. J., Mangione, C. M., Cabana, M., Caughey, A. B., Davis, E. M., Donahue, K. E., Doubeni, C. A., Krist, A. H., Kubik, M., Li, L., Ogedegbe, G., Pbert, L., Silverstein, M., Simon, M. A., Stevermer, J., Tseng, C. W., & Wong, J. B. (2021). Screening for Chlamydia and Gonorrhea: US Preventive Services Task Force Recommendation Statement. *JAMA*, 326(10), 949-956. <https://doi.org/10.1001/jama.2021.14081>
- de Vries, H. J. C., de Barbeyrac, B., de Vrieze, N. H. N., Viset, J. D., White, J. A., Vall-Mayans, M., & Unemo, M. (2019). 2019 European guideline on the management of lymphogranuloma venereum. *J Eur Acad Dermatol Venereol*, 33(10), 1821-1828. <https://doi.org/10.1111/jdv.15729>
- Dykewicz, C. A., Jaffe, H. W., & Kaplan, J. E. (2000). *Guidelines for Preventing Opportunistic Infections Among Hematopoietic Stem Cell Transplant Recipients*. <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr4910a1.htm>
- FDA. (2012a, 12/27/2012). *501(k) Premarket Notification Xpert CT/NG*. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=K121710>

- FDA. (2012b, 12/27/2012). *510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY K121710*.  
[https://www.accessdata.fda.gov/cdrh\\_docs/reviews/K121710.pdf](https://www.accessdata.fda.gov/cdrh_docs/reviews/K121710.pdf)
- FDA. (2019a, 05/23/2019). *501(k) Premarket Notification Xpert CT/NG, GeneXpert Dx System, GeneXpert Infinity-48s and GeneXpert Infinity-80 Systems, GeneXpert Infinity-48 System, Xpert Vaginal/Endocervical Specimen Collection, Xpert Urine Specimen Collection Kit, Xpert Swab Specimen Collection Kit*.  
<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm?ID=K190441>
- FDA. (2019b, 05/23/2019). *510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY K190441*.  
[https://www.accessdata.fda.gov/cdrh\\_docs/reviews/K190441.pdf](https://www.accessdata.fda.gov/cdrh_docs/reviews/K190441.pdf)
- FDA. (2021, 07/26/2021). *BD ONCLARITY HPV ASSAY*. U.S. Food & Drug Administration.  
<https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=391601>
- Feldman, S., & Crum, C. P. (2024, June 24). *Cervical cancer screening tests: Techniques for cervical cytology and human papillomavirus testing*.  
<https://www.uptodate.com/contents/cervical-cancer-screening-tests-techniques-for-cervical-cytology-and-human-papillomavirus-testing>
- Gaydos, C. A., Ako, M. C., Lewis, M., Hsieh, Y. H., Rothman, R. E., & Dugas, A. F. (2019). Use of a Rapid Diagnostic for Chlamydia trachomatis and Neisseria gonorrhoeae for Women in the Emergency Department Can Improve Clinical Management: Report of a Randomized Clinical Trial. *Ann Emerg Med*, 74(1), 36-44.  
<https://doi.org/10.1016/j.annemergmed.2018.09.012>
- Ghanem, K. G. (2024, July 8). *Clinical manifestations and diagnosis of Neisseria gonorrhoeae infection in adults and adolescents*. <https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-neisseria-gonorrhoeae-infection-in-adults-and-adolescents>
- Ghanem, K. G., & Tuddenham, S. (2024, March 15). *Screening for sexually transmitted infections*. Wolters Kluwer. Retrieved 06/30/2022 from  
<https://www.uptodate.com/contents/screening-for-sexually-transmitted-infections>
- Gilson, R., Nugent, D., Werner, R. N., Ballesteros, J., & Ross, J. (2020). 2019 IUSTI-Europe guideline for the management of anogenital warts. *J Eur Acad Dermatol Venereol*, 34(8), 1644-1653. <https://doi.org/10.1111/jdv.16522>
- Glass, N., Nelson, Heidi D. (2021). *Screening for Genital Herpes Simplex: A Brief Update for the U.S. Preventive Services Task Force*.  
<https://www.uspreventiveservicestaskforce.org/Home/GetFile/1/733/herpesup/pdf>
- Golden, M., O'Donnell, M., Lukehart, S., Swenson, P., Hovey, P., Godornes, C., Romano, S., & Getman, D. (2019). Treponema pallidum Nucleic Acid Amplification Testing To Augment Syphilis Screening among Men Who Have Sex with Men. *J Clin Microbiol*, 57(8).  
<https://doi.org/10.1128/jcm.00572-19>
- Goldstein, E., Martinez-García, L., Obermeier, M., Glass, A., Krügel, M., Maree, L., Gunson, R., Onelia, F., Pacenti, M., & Nelson, K. S. (2021). Simultaneous identification of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, and Trichomonas vaginalis—multicenter evaluation of the Alinity m STI assay. *Journal of Laboratory Medicine*, 45(4-5), 213-223.
- Guenat, D., Launay, S., Riethmuller, D., Mougin, C., & Pretet, J. L. (2016). Validation of Novaprep((R)) HQ+ liquid-based cytology medium for high-risk human papillomavirus detection by hc2. *Infect Agent Cancer*, 11, 41. <https://doi.org/10.1186/s13027-016-0092-7>



- Guy, R. J., Causer, L. M., Klausner, J. D., Unemo, M., Toskin, I., Azzini, A. M., & Peeling, R. W. (2017). Performance and operational characteristics of point-of-care tests for the diagnosis of urogenital gonococcal infections. *Sex Transm Infect*, 93(S4), S16-s21. <https://doi.org/10.1136/sextrans-2017-053192>
- Hicks, C. B., & Clement, M. (2022, September 27). *Syphilis: Screening and diagnostic testing*. <https://www.uptodate.com/contents/syphilis-screening-and-diagnostic-testing>
- Hicks, C. B., & Clement, M. (2023, December 20). *Syphilis: Epidemiology, pathophysiology, and clinical manifestations in patients without HIV*. <https://www.uptodate.com/contents/syphilis-epidemiology-pathophysiology-and-clinical-manifestations-in-patients-without-hiv>
- Hsu, K. (2024, May 1). *Clinical manifestations and diagnosis of Chlamydia trachomatis infections*. <https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-chlamydia-trachomatis-infections>
- Janier, M., Hegyi, V., Dupin, N., Unemo, M., Tiplica, G. S., Potocnik, M., French, P., & Patel, R. (2014). 2014 European guideline on the management of syphilis. *J Eur Acad Dermatol Venereol*, 28(12), 1581-1593. <https://doi.org/10.1111/jdv.12734>
- Janier, M., Unemo, M., Dupin, N., Tiplica, G. S., Potocnik, M., & Patel, R. (2020). 2020 European guideline on the management of syphilis. *Acta Clin Belg*. <https://doi.org/10.1080/17843286.2020.1773112>
- Juarez-Figueroa, L., Uribe-Salas, F., Garcia-Cisneros, S., Olamendi-Portugal, M., & Conde-Glez, C. J. (2007). Evaluation of a rapid strip and a particle agglutination tests for syphilis diagnosis. *Diagn Microbiol Infect Dis*, 59(2), 123-126. <https://doi.org/10.1016/j.diagmicrobio.2007.04.008>
- Kelly, H., Coltart, C. E. M., Pant Pai, N., Klausner, J. D., Unemo, M., Toskin, I., & Peeling, R. W. (2017). Systematic reviews of point-of-care tests for the diagnosis of urogenital Chlamydia trachomatis infections. *Sex Transm Infect*, 93(S4), S22-s30. <https://doi.org/10.1136/sextrans-2016-053067>
- Kingston, M., French, P., Higgins, S., McQuillan, O., Sukthankar, A., Stott, C., McBrien, B., Tipple, C., Turner, A., Sullivan, A. K., Radcliffe, K., Cousins, D., FitzGerald, M., Fisher, M., Grover, D., Higgins, S., Kingston, M., Rayment, M., & Sullivan, A. (2016). UK national guidelines on the management of syphilis 2015. *Int J STD AIDS*, 27(6), 421-446. <https://doi.org/10.1177/0956462415624059>
- Lanjouw, E., Ouburg, S., de Vries, H. J., Stary, A., Radcliffe, K., & Unemo, M. (2016). 2015 European guideline on the management of Chlamydia trachomatis infections. *Int J STD AIDS*, 27(5), 333-348. <https://doi.org/10.1177/0956462415618837>
- LeFevre, M. L. (2014). Screening for Chlamydia and gonorrhea: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*, 161(12), 902-910. <https://doi.org/10.7326/m14-1981>
- Liu, T. Y., Xie, R., Luo, L., Reilly, K. H., He, C., Lin, Y. Z., Chen, G., Zheng, X. W., Zhang, L. L., & Wang, H. B. (2014). Diagnostic validity of human papillomavirus E6/E7 mRNA test in cervical cytological samples. *J Virol Methods*, 196, 120-125. <https://doi.org/10.1016/j.jviromet.2013.10.032>
- Marcell, A. V., & Health, M. T. C. f. F. P. a. R. (2014). *Preventive Male Sexual and Reproductive Health Care: Recommendations for Clinical Practice*. U.S. Department of Health and Human Services. Retrieved 07/12/2018 from <http://content.guidelinecentral.com/guideline/get/pdf/2787>



- Moyer, V. A. (2014). Screening for oral cancer: U.S. preventive services task force recommendation statement. *Ann Intern Med*, 160(1), 55-60. <https://doi.org/10.7326/M13-2568>
- Murray, P., Braverman, P., Adelman, W., Breuner, C., Levine, D., Marcell, A. V., PJ, M., O'Brien, R., & Burstein, G. (2014). Screening for nonviral sexually transmitted infections in adolescents and young adults. *Pediatrics*, 134(1), e302-311. <https://doi.org/10.1542/peds.2014-1024>
- NCCN. (2023a, December 20). *NCCN Clinical Practice Guidelines in Oncology Anal Carcinoma*. [https://www.nccn.org/professionals/physician\\_gls/pdf/anal.pdf](https://www.nccn.org/professionals/physician_gls/pdf/anal.pdf)
- NCCN. (2023b, October 25). *NCCN Clinical Practice Guidelines in Oncology Penile Cancer*. Retrieved 06/30/2022 from [https://www.nccn.org/professionals/physician\\_gls/pdf/penile.pdf](https://www.nccn.org/professionals/physician_gls/pdf/penile.pdf)
- NCCN. (2024a, May 6). *NCCN Clinical Practice Guidelines in Oncology Cervical Cancer*. Retrieved 06/30/2022 from [https://www.nccn.org/professionals/physician\\_gls/pdf/cervical.pdf](https://www.nccn.org/professionals/physician_gls/pdf/cervical.pdf)
- NCCN. (2024b, May 1). *NCCN Clinical Practice Guidelines in Oncology Head and Neck Cancers*. Retrieved 06/30/2022 from [https://www.nccn.org/professionals/physician\\_gls/pdf/head-and-neck.pdf](https://www.nccn.org/professionals/physician_gls/pdf/head-and-neck.pdf)
- NCCN. (2024c, May 1). *NCCN Clinical Practice Guidelines in Oncology Vulvar Cancer (Squamous Cell Carcinoma)*. Retrieved 06/30/2022 from [https://www.nccn.org/professionals/physician\\_gls/pdf/vulvar.pdf](https://www.nccn.org/professionals/physician_gls/pdf/vulvar.pdf)
- NCCN. (2024d, April 29). *NCCN Clinical Practice Guidelines Occult Primary (Cancer of Unknown Primary [CUP])*. Retrieved 06/30/2022 from [https://www.nccn.org/professionals/physician\\_gls/pdf/occult.pdf](https://www.nccn.org/professionals/physician_gls/pdf/occult.pdf)
- NICE. (2018). National Institute for Health and Care Excellence: Clinical Guidelines. In *Cancer of the Upper Aerodigestive Tract: Assessment and Management in People Aged 16 and Over*. National Institute for Health and Care Excellence (UK) Copyright (c) National Collaborating Centre for Cancer. <https://www.nice.org.uk/guidance/ng36/evidence/full-guideline-2307980269>
- Nwokolo, N. C., Dragovic, B., Patel, S., Tong, C. Y., Barker, G., & Radcliffe, K. (2016). 2015 UK national guideline for the management of infection with Chlamydia trachomatis. *Int J STD AIDS*, 27(4), 251-267. <https://doi.org/10.1177/0956462415615443>
- Palefsky, J. M. (2024, July 11). *Human papillomavirus infections: Epidemiology and disease associations*. <https://www.uptodate.com/contents/human-papillomavirus-infections-epidemiology-and-disease-associations>
- Papp, J. R., Schachter, J., Gaydos, C. A., & Van Der Pol, B. (2014). Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae--2014. *MMWR Recomm Rep*, 63(Rr-02), 1-19. <https://www.cdc.gov/mmwr/pdf/rr/rr6302.pdf>
- Patel, R., Green, J., Clarke, E., Seneviratne, K., Abbt, N., Evans, C., Bickford, J., Nicholson, M., O'Farrell, N., Barton, S., FitzGerald, M., & Foley, E. (2015). 2014 UK national guideline for the management of anogenital herpes. *Int J STD AIDS*, 26(11), 763-776. <https://doi.org/10.1177/0956462415580512>
- Patel, R., Kennedy, O. J., Clarke, E., Geretti, A., Nilsen, A., Lautenschlager, S., Green, J., Donders, G., van der Meijden, W., Gomberg, M., Moi, H., & Foley, E. (2017). 2017 European guidelines for the management of genital herpes. *Int J STD AIDS*, 28(14), 1366-1379. <https://doi.org/10.1177/0956462417727194>

- Pham, M. D., Wise, A., Garcia, M. L., Van, H., Zheng, S., Mohamed, Y., Han, Y., Wei, W. H., Yin, Y. P., Chen, X. S., Dimech, W., Braniff, S., Technau, K. G., Luchters, S., & Anderson, D. A. (2020). Improving the coverage and accuracy of syphilis testing: The development of a novel rapid, point-of-care test for confirmatory testing of active syphilis infection and its early evaluation in China and South Africa. *EClinicalMedicine*, 24, 100440. <https://doi.org/10.1016/j.eclinm.2020.100440>
- Riley, L. E., & Wald, A. (2022, 02/10/2022). *Genital herpes simplex virus infection and pregnancy*. <https://www.uptodate.com/contents/genital-herpes-simplex-virus-infection-and-pregnancy>
- Society, C. P. (2024, March 28). *Diagnosis and management of congenital syphilis – Avoiding missed opportunities*. Canadian Paediatric Society. <https://www.cps.ca/en/documents/position/congenital-syphilis>
- Tsang, R. S., Martin, I. E., Lau, A., & Sawatzky, P. (2007). Serological diagnosis of syphilis: comparison of the Trep-Chek IgG enzyme immunoassay with other screening and confirmatory tests. *FEMS Immunol Med Microbiol*, 51(1), 118-124. <https://doi.org/10.1111/j.1574-695X.2007.00289.x>
- Tshomo, U., Franceschi, S., Tshoke, T., Tobgay, T., Baussano, I., Tenet, V., Snijders, P. J., Gheit, T., Tommasino, M., Vorsters, A., & Clifford, G. M. (2017). Evaluation of the performance of Human Papillomavirus testing in paired urine and clinician-collected cervical samples among women aged over 30 years in Bhutan. *Virol J*, 14(1), 74. <https://doi.org/10.1186/s12985-017-0744-2>
- Ullmann, A. J., Schmidt-Hieber, M., Bertz, H., Heinz, W. J., Kiehl, M., Kruger, W., Mousset, S., Neuburger, S., Neumann, S., Penack, O., Silling, G., Vehreschild, J. J., Einsele, H., Maschmeyer, G., Infectious Diseases Working Party of the German Society for, H., Medical, O., & the, D.-K. (2016). Infectious diseases in allogeneic haematopoietic stem cell transplantation: prevention and prophylaxis strategy guidelines 2016. *Ann Hematol*, 95(9), 1435-1455. <https://doi.org/10.1007/s00277-016-2711-1>
- Unemo, M. (2020). 2020 European guideline on the diagnosis and treatment of gonorrhoea in adults. *Int J STD AIDS*. <https://iusti.org/wp-content/uploads/2020/10/IUSTI-Gonorrhoea-2020.pdf>
- USPSTF. (2019). Preexposure Prophylaxis for the Prevention of HIV Infection: US Preventive Services Task Force Recommendation Statement. *JAMA*, 321(22), 2203-2213. <https://doi.org/10.1001/jama.2019.6390>
- USPSTF. (2023, February 14). *Genital Herpes Infection: Serologic Screening*. <https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/genital-herpes-serologic-screening>
- White, J., O'Farrell, N., & Daniels, D. (2013). 2013 UK National Guideline for the management of lymphogranuloma venereum: Clinical Effectiveness Group of the British Association for Sexual Health and HIV (CEG/BASHH) Guideline development group. *Int J STD AIDS*, 24(8), 593-601. <https://doi.org/10.1177/0956462413482811>
- Wong, E. H., Klausner, J. D., Caguin-Grygiel, G., Madayag, C., Barber, K. O., Qiu, J. S., Liska, S., & Pandori, M. W. (2011). Evaluation of an IgM/IgG sensitive enzyme immunoassay and the utility of index values for the screening of syphilis infection in a high-risk population. *Sex Transm Dis*, 38(6), 528-532. <https://doi.org/10.1097/OLQ.0b013e318205491a>
- Workowski, K. A., & Bolan, G. A. (2015). Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep*, 64(Rr-03), 1-137. <https://pubmed.ncbi.nlm.nih.gov/26042815/>

- Yao, Y. L., Tian, Q. F., Cheng, B., Cheng, Y. F., Ye, J., & Lu, W. G. (2017). Human papillomavirus (HPV) E6/E7 mRNA detection in cervical exfoliated cells: a potential triage for HPV-positive women. *J Zhejiang Univ Sci B*, 18(3), 256-262. <https://doi.org/10.1631/jzus.B1600288>
- Zhiyan, L., Meiling, W., Ping, L., Jinhua, D., Zhenlin, Y., & Zhenru, F. (2015). Consistency Between Treponema pallidum Particle Agglutination Assay and Architect Chemiluminescent Microparticle Immunoassay and Characterization of Inconsistent Samples. *J Clin Lab Anal*, 29(4), 281-284. <https://doi.org/10.1002/jcla.21765>

## X. Revision History

Revision Date	Summary of Changes
09/04/2024	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)
06/19/2024	Off-cycle coding modification: Added CPT code 0455U, 0463U (effective date 07/01/2024) Removed CPT code 0353U (effective date 07/01/2024)
03/06/2024	Off-cycle coding modification: Removed CPT code 0354U (effective date 04/01/2024)