The ABCs of STIs:
An Update on Sexually Transmitted Infections

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BACKGROUND: Sexually transmitted infections (STIs) are spread primarily through sexual contact and are a major cause of morbidity and mortality worldwide. Once identified, some STIs can be cured following appropriate therapy; for others, suppressive regimens and approaches to prevent ongoing transmission are important. The incidence of many common STIs is increasing in the US as well as worldwide, and hundreds of millions of people are currently infected. Laboratory testing plays a major role in the diagnosis and treatment of STIs, and clinical laboratorians should be familiar with the current guidelines and methods for testing.

CONTENT: Accurate and sensitive methods to diagnose STIs are essential to direct appropriate antimicrobial therapy and interrupt the cycle of disease transmission. This review summarizes laboratory testing for common bacterial, viral, and parasitic causes of STIs. Disease manifestations reviewed include cervicitis and urethritis, genital ulcerative disease, human immunodeficiency virus, viral hepatitis, human papilloma virus, and vaginitis. Recent advancements in the recognition and management of STIs, including updates to diagnostic algorithms, advances in testing methods, and emerging challenges with antimicrobial resistance, are summarized.

SUMMARY: Diagnostic methods and therapeutic guidelines for STIs are rapidly evolving. In combination with changing epidemiology, the development of novel therapeutics, and advancements in diagnostic methods, this has resulted in changing practices in laboratory testing and, subsequently, management of disease. Molecular methods have facilitated personalized therapy and follow-up regimens targeted for individual types or strains of some STIs.

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Over 100 million people in the US have a sexually transmitted infection (STI),[2] and almost 20 million new infections occur annually. The financial burden of STIs is substantial, at an estimated medical cost of $16 billion a year (1). While many of these diseases are preventable and treatable, the morbidity and mortality associated with STIs remain high. Antibiotic resistance is rapidly emerging, and the incidence of many common STIs is rising for the first time in a decade. However, new techniques in molecular diagnostics and the development of novel therapeutics have contributed to more rapid diagnosis and effective treatment. This review describes aspects of several STIs that are important to clinical laboratorians. A summary of the diseases and agents described can be found in Table 1.

Cervicitis and Urethritis

Inflammation of the cervix is characterized by a purulent endocervical exudate and easily induced cervical bleeding. Symptoms of urethritis include dysuria, urethral pruritis, and discharge. Cervicitis and urethritis are most commonly triggered by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. Additionally, bacterial infection with *Mycoplasma genitalium* is associated with urethritis.

CHLAMYDIA AND GONORRHEA
C. trachomatis and *N. gonorrhoeae* are gram-negative intracellular bacteria that are the most commonly reported causes of sexually transmitted disease in the US. Chlamydial infection is most prevalent in people ≤24 years old, with about twice as many infections reported in females compared with males (2). Although many infections are asymptomatic, there are severe sequelae of chlamydial and gonococcal infections, including pelvic inflammatory disease, ectopic pregnancy, and infertility.

Table 1. Clinical features and screening recommendations for common STIs.

Disease	Symptoms	Screening recommendations	Optimal testing method	Optimal specimen type	Reference
Chlamydia and gonorrhea	Cervical inflammation, dysuria, pruritus, and discharge	Females ≤25 years old, annually; MSM or young males in high-prevalence areas, annually	NAAT	Females: provider- or self-collected vaginal swab, first-void urine; males: urethral, rectal, or throat swab, first-void urine	CDC (16)
Syphilis	Primary: painless ulcer on genitals, rectum, or mouth; secondary: rash, wart-like sores, systemic symptoms; latent: symptomless stage of untreated syphilis; tertiary: organ damage	No routine screening; pregnant women: first prenatal visit; MSM: annually	RPR; confirmation: FTA-Abs or TP-PA	Serum	CDC (2); US Preventive Services Task Force (30)
HSV	Ulcers on the mouth, genitals, or rectum; may present with systemic symptoms	No routine screening; testing should be considered in men or women who present with STI	NAAT	Swab from the leading edge of active lesion	LeGoff et al. (42)
HIV	Acute: flu-like illness; chronic: AIDS, opportunistic infections	All people ages 13–64 years: one-time screening using opt-out approach; pregnant women: first prenatal visit; MSM: annually	Fourth-generation antibody/antigen testing	Serum	Cornett and Kim (51), CDC (55)
HBV	Abdominal pain, malaise, jaundice, and dark urine	No routine screening; pregnant women: first prenatal visit; MSM and others at increased risk	HBsAg	Serum	Workowski and Bolan (20), LeFevre (64)
HCV	Abdominal pain, malaise, jaundice, and dark urine; chronic: asymptomatic	One-time screening for all people born 1945–1965	HCV antibody	Serum	Kamili et al. (76), CDC (77)
HPV	Usually asymptomatic and self-limiting; high-risk subtypes linked to cervical and rectal neoplasias	Females: begin screening at 21 years of age	Cervical cytology by Pap smear; HPV NAAT (>30 years of age)	Cervical	Moyer (89)
Bacterial vaginosis	Abnormal vaginal discharge, itching, or odor	No routine screening	Optimal testing method is evolving	Vaginal	Workowski and Bolan (20), Brotman et al. (96)
Trichomoniasis	Asymptomatic; may have diffuse yellow-green discharge and vulvar irritation	No routine screening	NAAT	Vaginal, cervical, or urine	Van Der Pol (103)

Infections are transmitted through vaginal, oral, or anal intercourse. Additionally, *N. gonorrhoeae* is a cause of ocular infection, most commonly in neonates born to infected mothers. Effective prophylaxis measures have greatly decreased the incidence of neonatal gonococcal infection in the US, but transmission is still frequent in developing countries with high rates of untreated infection in pregnant women *(3)*.

Screening. The CDC recommends that all sexually active females <25 years of age be screened annually for chlamydial and gonococcal infection *(4)*. Additionally, all pregnant women <25 years old should be screened for *Chlamydia* and gonorrhea during the initial prenatal visit. Retesting in the third trimester should be considered for women at increased risk for *Chlamydia* to lessen the likelihood of birth complications and perinatal infection of a neonate *(2)*.

Screening of young males is recommended in areas of high prevalence and for men who have sex with men (MSM) *(5)*. The rate of reported cases of chlamydial and gonococcal infections among men has increased in recent years *(6)*. This may be a result of changes in screening practices, as screening of extragenital sites is now recommended owing to the high prevalence of pharyngeal and rectal *Chlamydia* and gonorrhea among MSM *(7–9)*.

The specimen of choice for screening females for urogenital infection is a vaginal swab that is provider- or self-collected in a clinical setting. The provision of self-collection has increased screening rates among women, and studies have demonstrated comparable sensitivity and specificity of these specimens to provider-collected samples *(10–12)*. Other acceptable specimens include first-catch urine or an endocervical swab. Urethral and first-catch urine samples are used to screen men for urogenital *Chlamydia* and gonococcal infection. Testing for both pathogens is available by nucleic acid amplification tests (NAATs); this approach has several advantages over culture and antigen testing. NAATs have exhibited superior diagnostic and analytical sensitivity for the detection of chlamydial and gonococcal infections *(13–15)*. Most commercially available assays are approved by the US Food and Drug Administration (FDA) for vaginal, urethral, cervical, and urine specimens. NAATs are not FDA cleared for throat and rectal specimens, but recommendations for screening these sites have prompted many laboratories to validate throat and rectal swabs for NAATs *(16)*.

Although NAATs demonstrate improved sensitivity compared with cultures of *N. gonorrhoeae* and *C. trachomatis*, there is an infrequent but important need for culturing these pathogens. Currently, culture is recommended in cases of sexual assault in boys and extragenital exposure in girls. In addition, some laboratories must maintain the ability to culture *C. trachomatis* and *N. gon-*

orrhoeae to monitor trends in epidemiology and antibiotic resistance patterns *(16)*. Culture of *C. trachomatis* requires cell culture to cultivate the organism *(15)*. Invasive specimen collection is necessary to acquire sufficient epithelial cells, and samples must be processed and inoculated onto a cell monolayer without delay. Infected cells are incubated with chlamydial antibodies to detect the presence of the organism. This process is technically complex, and methods are highly variable among laboratories *(17)*. Therefore, culture is not practical for most clinical laboratories and is typically reserved for public health facilities. Culture of *N. gonorrhoeae* can be difficult due to the fastidious nature of the organism. Specimens should be collected on swabs with plastic or metal shafts owing to possible inhibitory effects of wood or cotton on the growth of bacteria, and specialized transport medium is required to preserve organism viability. Additionally, selective and enriched growth media and incubation in an increased CO_2 environment are required for isolation.

Treatment. Treatment of chlamydial and gonococcal infection is critical to prevent further transmission to a partner or neonate or possible sequelae such as reproductive complications. The recommended treatment for chlamydial infections is azithromycin or doxycycline, which have been shown to be equally effective in eradication of infection *(18)*.

Treatment of gonococcal infection has evolved recently due to increasing concerns about antibiotic-resistant strains of *N. gonorrhoeae*. A recent increase in the incidence of tetracycline resistance and strains with increased minimum inhibitory concentrations to cefixime has prompted the CDC to recommend combination therapy with ceftriaxone and azithromycin *(2, 19, 20)*, which is also effective against uncomplicated chlamydial coinfection. If treatment failure is observed after this regimen, antimicrobial susceptibility testing (AST) of the *N. gonorrhoeae* strain should be performed.

MYCOPLASMA GENITALIUM

Approximately 20% of nongonococcal and nonchlamydial cases of male urethritis can be attributed to the bacterium *M. genitalium*, which may be sexually transmitted *(21)*. The pathogenesis of *M. genitalium* in women is unclear, although the organism is found more often in women with cervicitis than those without *(22, 23)*.

Diagnostic testing for *M. genitalium* infection can be a challenge. The organism can take up to 6 months to grow in culture, and very few laboratories can culture clinical isolates. Therefore, NAAT is the preferred method of testing, although there are currently no FDA-approved diagnostic assays. Thus, the organism may be underdiagnosed in people with persistent or recurrent nongonococcal urethritis.

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As a result of these diagnostic challenges, the decision to treat a suspected case of *M. genitalium* is largely based on symptomology and exclusion of other causes of infection. Studies have shown that doxycycline is relatively ineffective against *M. genitalium*, whereas azithromycin therapy has been more successful *(24, 25)*. However, resistance to azithromycin is rapidly emerging, and an alternative regimen consisting of treatment with moxifloxacin has been effective in a small number of cases in which previous treatment measures have failed *(26, 27)*.

Genital Ulcerative Disease

Genital ulcers can have infectious or noninfectious etiologies. Most cases in the US are caused by sexual transmission of herpes simplex virus (HSV) or syphilis. Less prevalent infectious causes include chancroid (*Hemophilus ducreyi*), granuloma inguinale (*Klebsiella granulomatis*), and lymphogranuloma venereum (serovar L1, L2, L2a, or L3 of *Chlamydia trachomatis*). The presence of genital ulcers is associated with an increased risk of HIV acquisition and transmission *(28, 29)*. However, determining the cause of genital ulcers is often complicated by the fact that multiple etiologies may be present simultaneously.

SYPHILIS

Syphilis, which is caused by the bacterium *Treponema pallidum*, is divided into primary, secondary, and tertiary stages of infection, depending on clinical manifestation. Asymptomatic infection is known as latent syphilis and may be detected by serologic testing. In addition, *T. pallidum* can infect the central nervous system at any stage of infection, resulting in neurosyphilis.

Screening and diagnostic testing. Routine screening for syphilis is not recommended in the US, owing to the low positive predictive value of testing asymptomatic individuals *(30)*. Because the majority of primary and secondary syphilis cases are diagnosed in MSM, screening should be reserved for these individuals and others at high risk of infection *(2)*. Transplacental transmission of syphilis from mother to fetus can result in stillbirth, hydrops fetalis, or premature birth. Because of the severe morbidity associated with infection, routine syphilis screening is recommended for all pregnant women during the first prenatal visit and in the third trimester for women who are at high risk for infection, live in high prevalence areas, or were previously untested *(20)*.

Serologic testing methods for syphilis are categorized as treponemal specific or nontreponemal. Treponemal tests include the *T. pallidum* enzyme immunoassay (TP-EIA), the *T. pallidum* particle agglutination (TP-PA) test, and the fluorescent treponemal antibody absorption (FTA-Abs) test. These are typically confirmatory tests that detect antibodies against treponemal cellular components. Although they display very good sensitivity and specificity, they are labor intensive and cannot be used to follow disease, as they remain positive after successful treatment.

Nontreponemal tests, including venereal disease research laboratory (VDRL) and rapid plasma reagin (RPR) tests, detect antibodies to a cardiolipin-cholesterol-lecithin antigen produced in response to syphilis infection. Although these tests lack specificity, they are rapid and inexpensive and can be used to screen for active infection and monitor response to treatment. Dilution of samples should be considered in patients with high clinical suspicion for syphilis because of a potential prozone effect with RPR and VDRL tests *(31, 32)*. In most settings, nontreponemal testing is used for initial screening, and positive results are confirmed by treponemal-specific testing *(33)*.

The recent development of automated treponemal serologic immunoassays has created a paradigm shift in the algorithm for syphilis diagnostic testing. With the availability of high-throughput automated enzyme immunoassays and the lower cost of treponemal-specific testing, some laboratories have initiated a "reverse screening algorithm" in which initial testing consists of a treponemal-specific method, followed by nontreponemal testing *(34)*. However, the low prevalence of syphilis in the US in most patient populations and geographic regions has a detrimental effect on the positive predictive value of this algorithm, which can lead to uncertainty about patient management *(35)*.

HERPES SIMPLEX VIRUS

Genital herpes infection, which is caused by HSV type 1 (HSV-1) and HSV-2, is one of the most prevalent STIs worldwide. In the US, the overall seroprevalence of HSV-2 in adolescents and adults is 17% *(36)*. Although the seroprevalence of HSV-2 has declined in recent years, an increasing proportion of new infections has been attributed to HSV-1, particularly in young women and MSM *(37, 38)*. Many infections are unrecognized, because genital HSV infections are often asymptomatic or subclinical.

A clinical diagnosis of genital herpes is neither sensitive nor specific. Therefore, laboratory testing methods are routinely used to support the diagnosis. Viral culture was once the gold standard for HSV diagnosis, but as a result of the long turnaround time and reduced sensitivity of the method compared with molecular testing, viral culture for HSV has largely fallen out of favor *(39)*. Nucleic acid testing by PCR assay of a swab of a lesion is highly sensitive in an individual presenting with active lesions *(40, 41)*. However, latent infection cannot be ruled out in a patient with no active lesions and a negative result. At the time of preparation of this manuscript,
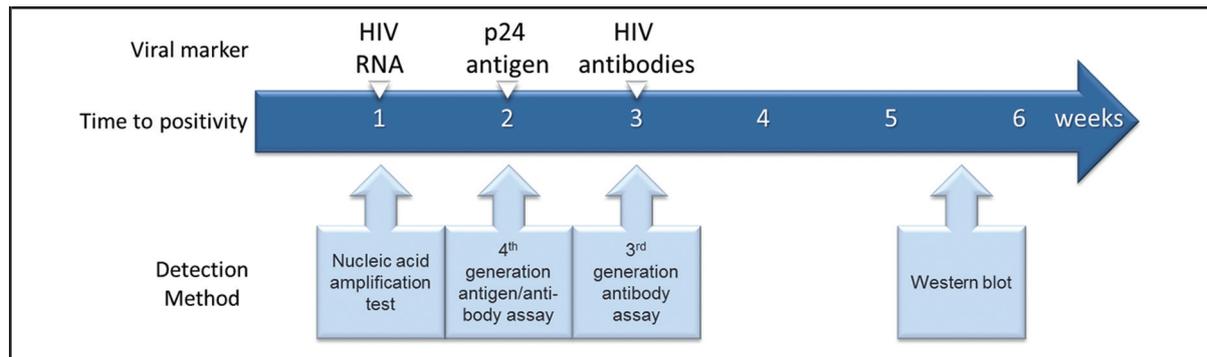
Fig. 1. Timeline of virologic and serologic markers of HIV infection.

Approximately 7–10 days after HIV exposure, viral RNA is detectable by nucleic acid amplification testing. Two weeks after infection, the expression of HIV p24 antigen is detectable by fourth-generation antigen/antibody assays. HIV-1 Western blots, which identify HIV-1 antibodies separated by electrophoresis, are not positive until 5–6 weeks after infection onset.

there are 3 FDA-cleared molecular assays for detection of HSV DNA in genital and oral lesions that provide rapid results with detection rates superior to viral culture *(42)*.

Serologic assays are available for type-specific testing of HSV-1 and -2. Antibodies develop within the first several weeks after infection and are present indefinitely. A positive IgG result is not diagnostic of active HSV infection. Furthermore, detection of IgM has no utility, as it does not correlate with recent infection and IgM testing is not type specific *(20)*.

Human Immunodeficiency Virus

Approximately 1.2 million people in the US and 35 million people worldwide are infected with HIV *(20, 43)*. In recent years, the rate of HIV diagnosis in the US has been stable at 15 per 100 000 population, with the overwhelming majority of new infections in men, particularly MSM *(44)*.

There are 2 types of HIV infection, HIV-1 and HIV-2. HIV-1 is the more prominent type and is estimated to cause approximately 95% of infections globally. Although HIV-2 infections have been reported worldwide, it is relatively rare compared to HIV-1. HIV-2 is endemic to West Africa and is an important cause of infection in areas with historic ties to this region, including Portugal, Spain, India, and Brazil *(45)*.

HIV is acquired through contact with infected bodily fluids, such as blood, semen, vaginal fluids, or breast milk, and most infections are acquired by sexual contact *(44)*. After a brief acute illness, HIV infection shifts to a chronic syndrome that progressively depletes CD4 T-lymphocytes, leading to AIDS and an increased risk of opportunistic infections.

SCREENING AND DIAGNOSIS

The CDC recommends that all people 13–64 years of age be screened for HIV with an "opt-out" approach, which means an individual is notified that testing for HIV will be performed unless the person declines *(46)*. The recommendation for expanded HIV testing should facilitate earlier diagnosis of HIV infections, which will decrease further spread of infection and permit prompt treatment with antiretroviral therapy before serious sequelae develop. In addition, HIV is an important cause of perinatal infection, and approximately 8700 babies are born to HIV-infected women each year in the US *(47)*. Therefore, the CDC recommends that all pregnant women be screened for HIV early in pregnancy, and retesting is recommended during the third trimester for women at high risk of acquiring HIV infection *(48, 49)*. Prenatal HIV screening has reduced perinatal HIV infection, as women who test positive can be started on antiretroviral therapy and managed appropriately during delivery to reduce the risk of transmission *(50)*.

The CDC has recently updated the laboratory testing algorithm for diagnosis of HIV. The first step in the algorithm is screening with a fourth-generation combined antibody/antigen immunoassay (IA), which combines serologic testing for both HIV-1 and HIV-2 and virologic testing for detection of the HIV p24 antigen. Previously implemented third-generation assays did not include p24 antigen detection. Therefore, testing with fourth-generation methods shortens the window for detection of acute infection from approximately 1 month with third-generation assays to 15–20 days, attributed to the ability to detect HIV infection before seroconversion (Fig. 1) *(51, 52)*. Evaluations of the diagnostic performance of combination assays have demonstrated sensitivity and specificity of 89%–99.9% and 99.5%, respec-

tively, for diagnosis of HIV, including acute infections (53, 54).

Several FDA-cleared rapid HIV tests are available that permit a preliminary diagnosis of HIV infection in <30 min. Most of these are third-generation assays. It is now recommended that all reactive rapid tests be confirmed by following the algorithm from the beginning and testing the sample with a laboratory-based fourth-generation antigen/antibody combination IA (55).

If the screening assay is nonreactive, no further testing is necessary. Specimens with a reactive result are tested in the second step of the algorithm with an IA that differentiates HIV-1 antibodies from HIV-2 antibodies (55). Differentiation of HIV types is important, because strains of HIV-2 are naturally resistant to many antiretroviral drugs and are managed differently (56). The differentiation IAs have several advantages over the Western blot confirmation method, including faster turnaround time, lower cost, and recognition of HIV-2 strains.

If the differentiation assay is nonreactive or indeterminate, the specimen is further tested with an HIV-1 NAAT to detect viral RNA. A reactive HIV-1 NAAT result is indicative of acute HIV-1 infection, whereas a negative result denotes a false positive on the screening test (55).

MANAGEMENT

Management of the HIV-infected patient is complicated and includes viral load monitoring, CD4 T cell counts, and HIV antiretroviral resistance testing. For further information on HIV management, we refer the reader to the HIV treatment guidelines (57).

Viral Hepatitis

Hepatitis may result from many infectious and noninfectious causes. Viral hepatitis most often induces a hepatocellular pattern of liver injury in which aspartate aminotransferase and alanine aminotransferase are markedly increased (i.e., >10–20 times the upper limit of the reference interval). Common symptoms of acute viral hepatitis include malaise, fever, nausea, vomiting, jaundice, and dark urine. Hepatitis B virus (HBV) is a well-known cause of sexually transmitted viral infection, whereas hepatitis C virus (HCV) is generally not efficiently transmitted through sexual contact (58, 59). However, recent reports have indicated an increasing incidence of HCV in MSM in the US and Europe (60–63). Therefore, the CDC considers sexual transmission of HCV an emerging issue.

HEPATITIS B VIRUS

Sexual transmission is thought to be the major route of HBV infection in developed countries. The virus is efficiently transmitted via mucosal exposure to infected blood or body fluids. Infections are usually self-limiting, but a small percentage of infected adults go on to develop chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Risk factors include sexual intercourse with multiple partners, anal sex, and intravenous drug use. HBV is highly endemic (>8%) in much of East Asia and sub-Saharan Africa, whereas prevalence is low (<2%) in the US (64).

Viral hepatitis occurs with the same frequency in pregnant women as would be expected in a comparable age group (65). Women who acquire hepatitis B late in pregnancy or who are chronic carriers are more likely to transmit the disease to their babies, with possible outcomes including fulminant hepatitis, mild hepatitis, or chronic hepatitis. As a result, all pregnant women should be screened for hepatitis B infection in the first trimester and at delivery if at high risk for HBV infection (66).

Diagnosis. Serologic testing is used to diagnose acute or chronic HBV (Fig. 2). In certain populations, such as transplant patients, serologic markers may be useful to categorize the risk for HBV reactivation after immune suppression. We refer the reader to the published guidelines on this topic for further information (67). Hepatitis B surface antigen (HepBsAg) and e antigen (HepBeAg) appear in the blood within weeks to months after infection onset and are likely to be present in a symptomatic patient seeking care. HepBsAg is present in both acute and chronic infections. Therefore, the presence of an IgM antibody directed against the hepatitis B core antigen (anti-HBc-IgM) is helpful in the differentiation of acute vs chronic infection (Table 2). Acutely infected individuals are anti-HBc total and IgM positive, whereas the anti-HBc-IgM is negative in chronic infection (Table 2). The decline of HepBeAg levels and subsequent rise in HepB e antibodies (HepBeAb) indicates that acute infection has resolved. In chronically infected individuals, the detection of HepBeAg in conjunction with HBV DNA suggests active viral replication and high infectivity. Antibody to HBsAg (anti-HBs) is produced in people with resolved infection and is the only serologic marker present in vaccinated individuals.

Prevention. Major efforts to vastly decrease the incidence of HBV infection include strategies aimed at increasing vaccination rates and preventing vertical transmission of HBV from a mother to her infant. The Advisory Committee on Immunization Practices (ACIP) recommends vaccination of infants at birth, of all previously unvaccinated children and adolescents, and of previously unvaccinated adults who are at increased risk of infection (68, 69). In 2010, hepatitis B vaccine coverage rates among adults 19–49 years old at high risk for infection and in health care personnel were 42% and 63.2%, respectively, far below the 90% target rate (70). Despite
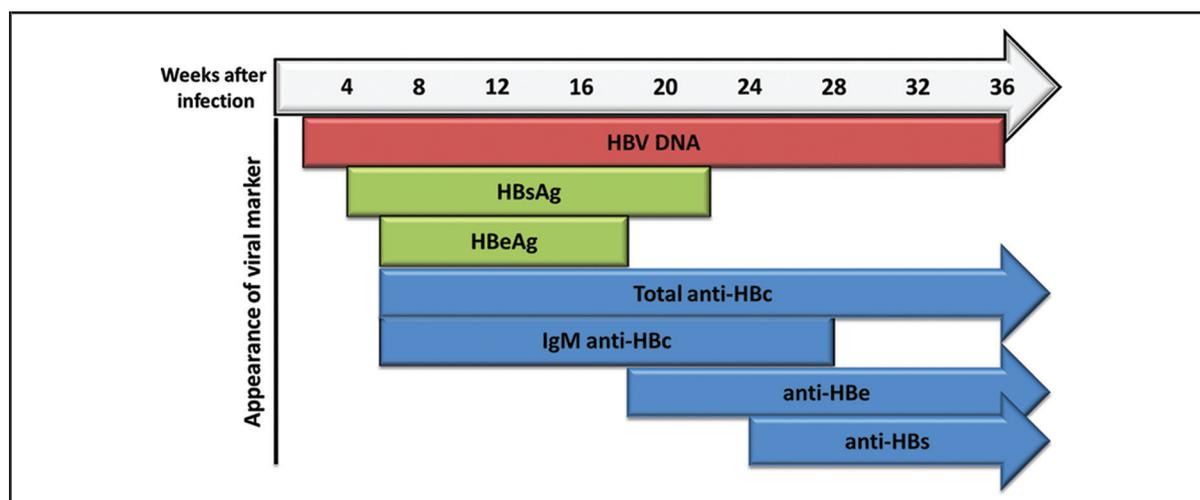
Fig. 2. Appearance of virologic and serologic markers in acute HBV infection.

The appearance of HBV markers occurs in a predictable pattern. HBV DNA (red bars) is detectable very early in infection and declines until approximately 36 weeks after infection onset. HBV antigens (green bars) appear within 4–6 weeks after exposure and serve as good markers of acute infection. HBV antibodies appear approximately 6 weeks after infection. IgG antibodies to the core and surface antigens generally persist for life and serve as a marker of chronic or past infection.

these low rates, the incidence of acute HBV infections has declined by 62% since the year 2000 *(71)*. Current guidelines for the treatment of HBV infection are reviewed elsewhere *(72)*.

HEPATITIS C VIRUS

More than 3 million people in the US are chronically infected with blood-borne HCV, and global prevalence is increasing worldwide. Chronic HCV infection causes considerable morbidity due to increased risk of liver cirrhosis, hepatocellular carcinoma, and liver failure. The association of HCV with high-risk sexual behaviors, such as sex with multiple partners, coinfection with other STIs, and traumatic sex, has led the CDC to include HCV infection as an emerging issue in STIs *(20)*.

Because there is no effective vaccine for HCV, current efforts are focused on effective screening measures and treatment of infected individuals, and several recent advances in these areas may improve the clinical outcomes of infected patients.

Screening. In the past, screening for HCV was recommended for individuals, such as intravenous drugs users, considered at high risk for HCV transmission. However, chronic HCV infection is frequently asymptomatic, and many infected individuals are unaware of their diagnosis *(73)*. Therefore, the CDC has recently updated screening guidelines to include one-time HCV antibody testing for any individual born between 1945 and 1965, even in the absence of identifiable risk factors. It has been demonstrated that people in this cohort are approximately 6 times more likely to have been exposed to HCV than adults in other age cohorts *(74, 75)*.

Table 2. Summary of interpretations of HBV virologic and serologic test results.

Hepatitis B status	DNA	HBsAg	HBeAg	Anti-HBc, total	Anti-HBc, IgM	Anti-HBe	Anti-HBs
Acute	+	+	+/−	+	+	−	−
Resolved	−	−	−	+	−	+	+
Chronic carrier state	−	+	−	+/−	−	+	−
Chronic active HBV	+	+	+	+/−	−	−	−
Chronic active HBV, pre-core mutant	+	+	−	+/−	−	+	−
Vaccinated	−	−	−	−	−	−	+

Review

Identification of HCV-infected individuals begins with an IA to detect HCV antibody, which permits detection as early as 10 weeks after exposure (76). Robust enzyme IAs, chemiluminescent IAs, and rapid IAs for HCV antibody are available for clinical testing. For a review of performance characteristics of these assays, we refer the reader to Kamili et al. (76). Historically, recombinant immunoblot assays (RIBAs) were used to distinguish a false-positive enzyme IA (EIA) result (RIBA negative) from an EIA positive due to a past infection (RIBA positive). However, this method is no longer widely used and is unavailable in the US. In a person with a negative antibody test but a high suspicion of HCV infection or anyone with a positive HCV antibody test, NAAT to detect HCV RNA is necessary to confirm a diagnosis of HCV infection (77). Once a diagnosis is confirmed, HCV genotype (1–6, with numerous subtypes) should be determined, as the specific molecular makeup of the virus has important implications for treatment options.

Treatment. The treatment of HCV infection is rapidly evolving from an interferon-based therapy to an interferon-free regimen that is based on direct-acting antiviral agents. These new therapeutics have markedly increased rates of sustained virologic response and more acceptable side effect profiles. Physicians have swiftly embraced therapy with direct-acting antiviral agents, so management of HCV for both clinicians and laboratorians is rapidly evolving (78–80).

Human Papilloma Virus

More than 100 subtypes of human papillomaviruses (HPVs) cause infection of human epithelial or mucosal surfaces, depending on tissue tropism. Of those subtypes, >40 can infect the genital tract. Subtypes 6 and 11 are most often associated with genital warts, whereas infection with high-risk subtypes including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 are linked to cervical and rectal neoplasia (81).

The burden of HPV infection is high in the US and worldwide, with an estimated overall prevalence of 10.4% (82). The prevalence of both low-risk and high-risk HPV subtypes is highest in women <30 years of age (2). Approximately half a million cases of cervical cancer are diagnosed annually. Of those, the high-risk HPV subtypes 16 and 18 account for 50% and 20% of cases, respectively (83).

HPV infection is usually asymptomatic and self-limiting. When symptoms are present, they may manifest as benign raised lesions in the genital area. Cervical cancers that arise from HPV infection may present as abnormal cervical cytology screening or with irregular vaginal bleeding. HPV is spread by contact with infected mucosal surfaces. Therefore, vaginal and anal intercourse are major risk factors for transmission of the virus (84).

SCREENING

The incidence of cervical cancer has steadily declined since the initiation of widespread screening in the US and other developed countries (85, 86). Despite this decline, a 2012 survey estimated that 11.4% of women had not been screened in the preceding 5 years (87). Beginning at 21 years of age, cervical cytological screening is recommended to detect invasive cervical cancer. Annual screening is no longer recommended. Instead, women should be screened every 3 years until age 65. At age 30 and beyond, screening may include testing with one of the several FDA-cleared high-risk HPV NAAT tests available that detect oncogenic subtypes of HPV. Specimens for HPV testing include the same swab used for cytology testing, a separate swab, or the residual fluid from liquid-based cytology testing (20). ThinPrep liquid-based cytology specimens are FDA cleared for HPV testing. SurePath cytology specimens, however, are not FDA cleared, owing to higher than acceptable rates of false-negative tests that may be due to degradation of HPV nucleic acid in the cytology fluid (88). FDA-cleared high-risk HPV NAATs detect oncogenic subtypes of HPV. See Table 3 for details on available HPV NAATs. Owing to the high negative predictive value of coscreening, women screened with Pap and HPV testing should be screened every 5 years (89, 90).

PREVENTION

Prevention of cervical cancer resulting from HPV infection is possible with vaccination. The ACIP recommends routine vaccination of girls and boys 11 and 12 years of age with one of several available HPV vaccines (91, 92). All 3 of these vaccines cover the oncogenic subtypes 16 and 18, and the quadrivalent vaccine additionally covers subtypes 6 and 11, which are the most common causes of genital warts. The 9-valent vaccine has recently been approved, which covers additional high-risk subtypes responsible for about 15% of cervical cancers (92). The quadrivalent and 9-valent vaccines are approved for use in males.

HPV vaccination has been recommended by the ACIP for adolescent girls since 2006 and adolescent boys since 2011. However, the number of individuals receiving HPV vaccination in the US has been below the target of 80% (93). Data from a CDC survey indicates that 57.3% of girls ages 13–17 years had received at least 1 of the 3 recommended doses of the vaccine, and only 37.6% had received all 3. Coverage of boys was even lower, with 34.6% having received at least 1 dose and 13.9% receiving all 3 (94).

Table 3. Currently available nucleic acid amplification tests for detection of human papilloma virus[a]

HPV NAAT	Serotypes detected	Method	Specimen	FDA indication
Digene Hybrid Capture 2 High-Risk HPV DNA Test (HC2)	Pooled subtypes including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68	DNA-based screening assay	Cervical specimens in PreservCyt solution collected with HC2 collection device or broom-type device	(1) Screening patients with ASC-US[b] cervical cytology results for referral to colposcopy; (2) screening for presence or absence of hrHPV in conjunction with cervical cytology in women > 30 years of age
Cervista HPV HR	Pooled subtypes 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 66, and 68	DNA-based screening assay	Cervical specimens in PreservCyt solution, collected with broom-type device or endocervical brush or spatula	(1) Screening patients with ASC-US cervical cytology results for referral to colposcopy; (2) screening for presence or absence of hrHPV in conjunction with cervical cytology in women >30 years of age
Cervista HPV 16/18	16, 18	DNA-based genotyping assay	Cervical specimens in PreservCyt solution, collected with broom-type device or endocervical brush or spatula	Assessing presence or absence of hrHPV types 16 and 18 adjunctively with Cervista HPV HR test in combination with cervical cytology (1) in women >30 years of age or (2) in patients with ASC-US cytology results
Aptima HPV hrHPV E6/E7	Pooled subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68	HPV E6/E7 mRNA-based screening assay	Cervical samples in PreservCyt solution collected with broom-type or cytobrush/spatula collection device	(1) Screening patients with ASC-US cervical cytology results for referral to colposcopy; (2) screening for presence or absence of hrHPV in conjunction with cervical cytology in women >30 years of age
Cobas HPV Test[c]	Individual identification of subtypes 16 and 18 and pooled detection of subtypes 1, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68	DNA-based screening assay with concurrent genotyping	Cervical specimens in PreservCyt solution collected with endocervical brush or spatula	Assessing women ≥21 years of age with ASC-US cervical cytology results for (1) referral to colposcopy or (2) presence or absence of hrHPV genotypes 16 and 18; screening women ≥30 years of age for (1) hrHPV types or (2) presence or absence of types 16 and 18

[a] For the most up-to-date information, always check the package insert for the assay of interest (108, 109).
[b] ASC-US, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papilloma virus subtype.
[c] At the time of writing, the Cobas HPV test is the only assay approved as a first-line primary screening test for cervical cancer.

Review

Vaginitis

Vaginitis is a common gynecologic condition, especially during a woman's reproductive years. It is characterized by abnormal vaginal discharge, itching, or odor. While most cases of vaginitis are caused by vulvovaginal candidiasis, many can be attributed to sexually transmitted infections caused by bacterial vaginosis and trichomoniasis.

BACTERIAL VAGINOSIS

Bacterial vaginosis (BV) occurs upon depletion of *Lactobacillus* species and overgrowth of other bacteria that results in a dysbiosis of the normal vaginal flora (95). BV is the most common cause of abnormal vaginal discharge in women presenting for clinical care. The cause of the microbial inequality that triggers BV is a subject of ongoing controversy, but women with BV are at increased risk of acquiring and transmitting other STIs (96, 97).

BV is often diagnosed by clinical criteria called Amsel criteria, which require the presence of at least 3 signs or symptoms, including the presence of clue cells (vaginal epithelial cells covered with adherent bacteria), characteristic vaginal discharge, a fishy odor, or vaginal fluid with a pH >4.5 (98). Although considered the historical gold standard, gram staining of vaginal discharge is infrequently used for BV diagnosis. The stain may reveal the presence of bacterial morphologies characteristically associated with BV. Compared to gram staining, the sensitivity and specificity of the Amsel criteria are 70% and 90%, respectively (99). In spite of this, the use of clinical criteria for diagnosis is more common because of the time, expense, and expertise required for the gram staining method. Current molecular testing methods for BV provide an incomplete picture for diagnosis and are focused on the presence or absence of a few BV-associated microorganisms. However, detection of these organisms alone is not adequate to establish the diagnosis, and results should be correlated with clinical signs and symptoms of BV. Because of the complex polymicrobial nature of BV, culture has no role in the diagnosis of infection.

Owing to the increased risk of acquisition of other STIs, treatment is advised for symptomatic women. The recommended regimen consists of topical or oral metronidazole or topical clindamycin (20).

TRICHOMONAS VAGINALIS

Trichomoniasis is an infection of the urogenital tract caused by the protozoan *Trichomonas vaginalis*. It is one of the most common nonviral causes of STI, with a prevalence of 3.1% in all women and 13.3% in black women in the US (2). Although most infections are asymptomatic, some women experience diffuse yellow-green discharge and vulvar irritation. The organism has been linked to adverse pregnancy outcomes, including increased risk of preterm birth, premature rupture of membranes, and delivery of an infant with decreased birth weight in infected women (100).

There is insufficient data to support routine screening for *T. vaginalis* infection, even in high-prevalence areas. Screening for trichomoniasis is not recommended as part of routine prenatal care in asymptomatic women (20, 101–103). However, because of possible adverse outcomes in pregnancy, pregnant women exhibiting symptoms of *T. vaginalis* infection should be evaluated and treated appropriately.

Diagnostic testing for trichomoniasis is usually done in conjunction with *Chlamydia* and gonorrhea testing. Detection of trichomonads by wet-mount microscopy is low cost and convenient and thus commonly used. However, this method has poor sensitivity, as the organism is reportedly detected in only 60%–70% of cases (104). The gold standard for detection of *T. vaginalis*, molecular testing by NAAT, has reported sensitivity and specificity of 95%–100% (105, 106). FDA-cleared NAAT assays are available that test vaginal, cervical, or urine specimens from women and, recently, urine specimens from men. Diagnostic sensitivity may be increased by following an algorithm in which a negative wet mount is followed by NAAT (107). In addition, less sensitive rapid antigen tests are available at the point of care that are FDA approved for vaginal secretions in women.

Conclusion

Diagnostic methods and therapeutic guidelines for STIs are rapidly evolving. This evolution, in combination with changing epidemiology, development of novel therapeutics, and advancements in diagnostic methods, has resulted in changing practices in laboratory testing and, subsequently, management of disease. Molecular methods have facilitated personalized therapy and follow-up regimens targeted for individual types or strains of some STIs. Thus, both awareness and understanding of testing methods are necessary for accurate and timely diagnosis of these important causes of infection.

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