

# Diagnostic tests for common STDs and HSV-2

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Recent years have witnessed steady levels of most sexually transmitted diseases (STDs), along with a significant resurgence in syphilis cases.<sup>1,2</sup> At the same time, diagnostic tests for STDs have increased in number and diversity. This article summarizes the most important standard tests for common sexually transmitted infections in adults, highlighting newer tests designed to address challenges in management and control of these infections.

Since infection with one STD is a marker of risk for contracting others, familiarity with the diagnostic modalities available for all such infections is important. Diagnostic testing is a critical tool for stemming spread of new infections. The invasiveness of diagnostic tests, their ease of use, ability to conduct them in alternative clinical and nonclinical settings, timing of results, and cost are important test factors that contribute to effective disease control. In some instances, newer diagnostic tests have replaced more labor-intensive and/or costlier techniques, such as bacterial culture and older assays necessitating daily preparation of reagents, nondisposable hardware, and intensively skilled staff for results interpretation. Increasingly, testing for STDs is conducted when and where the patient presents, such as in pre-natal and STD clinics. Such point-of-care testing minimizes mistreatment based on clinical diagnosis alone and addresses public health imperatives to recognize and treat STDs early, blunting further disease transmission.

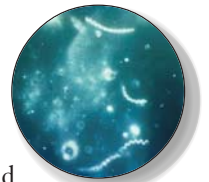
## Syphilis

Infection caused by the spirochete *Treponema pallidum* (*TP*) is diagnosed by direct examination of suspicious lesion material and conventional serologic tests for syphilis. The organism cannot be cultured routinely, and the gold-standard rabbit infectivity test is impractical to perform. The selection and timing of serologic tests is integral to recognition and appropriate management of syphilis and necessitates an understanding of the natural history of the disease. The emergence of rapid tests for syphilis offers promise for timelier identification, treatment, and interrupted spread of this infection, which is a significant cofactor in human immunodeficiency virus (HIV) susceptibility and transmission.<sup>3</sup>

Roughly one-third of individuals sexually exposed to a person with syphilis will become infected.<sup>4</sup> After exposure and incubation, the primary stage ensues with one or more typically painless ulcers (chancres) developing at the site of exposure, usually on the genitals or anus, with or without regional lymph-node enlargement. Such symptoms may appear within several days of exposure (mean, 21 days; range, 14 to 90 days). The chancre then resolves, even without treatment, in one to five weeks.<sup>4,5</sup> In the secondary stage, symptoms typically include mucosal lesions and rashes that may cover the palms and soles. Constitutional symptoms (e.g., sore throat, low-grade fever, malaise, muscle and joint aches, enlarged lymph nodes) also may be present. Less common symptoms and signs include oral mucous patches, *condylomata lata* (moist, wart-like papules occurring mostly in skin folds), and hair loss.

In latent syphilis, serologic evidence of infection is found despite absence of symptoms and signs of the primary and secondary (P&S) stages. Early latent syphilis is defined by the Centers for Disease Control and Prevention (CDC) as infection less than 12 months in duration.<sup>6</sup> Without evidence of acquisition in the prior year, infection is referred to as syphilis of unknown duration. During the early latent phase, relapses with secondary stage symptoms may occur.

Tertiary syphilis describes disease presenting with late manifestations, encompassing cardiovascular features such as aortitis with aneurysm formation, late neurologic sequelae, and formation of gummas (indolent, destructive lesions occurring in any organ but chiefly involving skin, bone, and liver). Late-stage manifestations may occur in one-third of untreated cases, and as long as 10 to 20 years after infection. Neurosyphilis, or central nervous system involvement by *TP*, is not a stage but rather a site of infec-



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### LEARNING OBJECTIVES

Upon completion of this article the reader will be able to:

1. Identify the most important standard test for common sexually transmitted infections.
2. Identify new tests for common sexually transmitted infections.
3. Discuss the use of these tests to address management and control of these infections.

Herpes capsid images courtesy of Hong Zhou, Ph.D., University of Texas Health Science Center, Department of Pathology and Laboratory Medicine

tion, where symptoms may manifest either earlier or later in the course of infection and may involve the visual and auditory systems prominently.

**Direct testing of clinical specimens.** *TP* may be detected using specimens collected noninvasively from skin and mucosal lesions suspicious for P&S syphilis using dark-field microscopy, with sensitivity of 75% to 80%, depending on specimen adequacy.<sup>7</sup> *TP*-specific staining of histologic specimens may also be performed, but sensitivity is diminished if concentration of treponemes is low. In clinical settings equipped with a dark-field microscope, the clinician may scrape the suspected syphilis lesion with a glass slide to prepare a wet mount for immediate examination. Identification of motile, corkscrew-shaped organisms appearing fluorescent green supports the diagnosis of syphilis. Specimens from oral lesions should *not* be examined using dark-field microscopy, as saprophytic, nonpathogenic treponemes may cause a false-positive result for *TP*.

Many clinics do not have dark-field microscopes and/or adequately trained staff to operate them. In these instances, smears from suspected syphilis lesions may be collected and submitted to the laboratory for fixation and immunostaining by the direct fluorescent antibody to *TP* (DFA-*TP*) method, which uses a fluorescein isothiocyanate-labeled globulin to detect *TP* antigen. Unlike direct dark-field examination, the DFA-*TP* employs a *TP*-specific conjugate; thus, specimens collected from oral lesions may be tested by this method. Sensitivity and specificity of DFA-*TP* approximate 100% using properly prepared specimens.<sup>8</sup>

A multiplex polymerase chain reaction (PCR) test for genital ulcer disease, or GUD, has been developed that simultaneously detects *TP*, *Haemophilus ducreyi* (the etiologic agent of chancroid), and herpes simplex virus (HSV) in ulcer material (Roche Diagnostics Corp., Basel, Switzerland). While its sensitivity for *TP* is excellent, approximating 95%, there are no plans to market this test in the United States.<sup>9</sup>

**Serologic testing for syphilis.** Routinely employed to diagnose and monitor treated syphilis are nontreponemal assays that use cardiolipin-, lecithin-, and cholesterol-containing antigen to measure antilipoidal IgM and IgG antibodies. The two most common such tests are the rapid plasma reagin (RPR) card and venereal disease research laboratory (VDRL) slide tests. By contrast, treponemal tests utilize antigen from *TP* or its components and often are used to confirm the results of nontreponemal tests in a two-step, reflex process. Treponemal tests include the fluorescent treponemal antibody absorption (FTA-Abs) test, the Serodia *TP* particle agglutination (TP-PA) test (Fujirebio America Inc., Fairfield, NJ), and its manufacturer's predecessor test, the microhemagglutination assay for antibodies to *TP*, or MHA-*TP*. The FTA-Abs requires a fluorescent microscope to detect adherence of the patient's serum antibody to *TP* antigen fixed to a slide. By contrast, the TP-PA is an agglutination assay using colored gelatin particle carriers sensitized with *TP* antigen.

Nontreponemal tests may be performed qualitatively or quantitatively, the latter enabling the clinician to follow serologic response to treatment. For quantitative tests, serum is diluted in a serial twofold fashion, and the last dilution in which the specimen is fully reactive is reported. Of significance to the laboratorian, the VDRL test requires daily preparation of an antigen suspension and use of reusable slides, whereas the RPR card test utilizes disposable plastic-coated cards.<sup>8</sup> Given variation in antigen preparation among the

commonly available nontreponemal tests, reactivity level is variable and, therefore, the tests cannot be interchanged in monitoring response to treatment.

**Sensitivity of serologic tests.** The sensitivity of both nontreponemal and treponemal tests varies with stage of infection. The sensitivity of nontreponemal tests increases with duration of infection, and ranges from approximately 75% to 85% in the primary stage to virtually 100% in the secondary stage.<sup>10</sup> Humoral antibodies typically are detectable one to four weeks after chancre formation. Since the sensitivity of nontreponemal tests is lower in the primary stage, a negative nontreponemal test in an individual with a genital lesion cannot exclude primary syphilis. In such patients, direct laboratory examination of suspicious lesions should be sought.

The sensitivity of treponemal tests continues to approximate 100% in late syphilis, in contrast to nontreponemal tests, which are more practical and cost-effective for initial screening but have diminished sensitivity in late syphilis. Despite the higher sensitivity of treponemal tests, data supporting their use for initial screening are limited. Nonetheless, it may be useful to employ both a nontreponemal and (nonreflexed) treponemal test to identify newly infected persons with suspicious lesions. For instance, in a recent evaluation of 39 dark-field confirmed syphilis cases,

VDRL was reactive in 30 (77%) whereas TP-PA was positive in 37 (95%). Using a strategy of initially obtaining both VDRL and TP-PA would have identified 100% of cases, compared to only 72% of cases using a conventional reflex strategy.<sup>11</sup>

Of special significance in the laboratory, the prozone phenomenon may lead to false-negative serologic test results, more often during the secondary stage. This reaction occurs when a high concentration of treponemal antigen does not permit detectable antigen-antibody complex formation. It may be overcome by dilution of the specimen. Clinicians should be reminded of the need to request specimen dilution in highly suspicious cases in which serologic tests are nonreactive. Finally, temperature of the laboratory (<73°F) may also contribute to false-negative nontreponemal test results.<sup>12</sup>

**Specificity of serologic tests.** Syphilis test specificity is a function of the test used and the population tested. False-positive nontreponemal results are known to occur in the setting of coexisting infection, autoimmune disease, and drug use, among other conditions. In the general population, false-positive results may occur at a rate of 1% to 2%.<sup>8</sup> In HIV-infected persons, nonspecific polyclonal B-cell activation may lead to false-positive nontreponemal results or higher test titers than in those uninfected with HIV.<sup>13,14</sup> While many have observed that biologic false-positive nontreponemal test titers typically are less than 1:8, such low titers may also occur in latent syphilis. From a public health perspective, positive nontreponemal tests, particularly with titers greater than 1:8, should be interpreted as indicating active infection, with interval testing to assess delayed seroreactivity of the confirmatory test.

False-positive treponemal test results also occur in the general population, though at a lower rate than that seen with nontreponemal tests. In these instances, techniques are available to help resolve the treponemal test status, involving processing of patient serum and/or use of an investigational Western blot assay specific for *TP* antibodies.<sup>8</sup> Rarely, false-positive treponemal tests may be due to infection with other nonvenereal treponemal infections like yaws, bejel, or pinta, particularly in persons from endemic areas.

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*Roughly one-third of individuals sexually exposed to a person with syphilis will become infected.*

**Laboratory diagnosis of neurosyphilis.** Laboratory diagnosis of neurosyphilis is based primarily on a reactive VDRL-CSF. When the VDRL is negative, the diagnosis is suggested by elevated white blood cells (WBC), with or without elevated protein concentration, in the absence of other known causes of these abnormalities. Use of secondary criteria is problematic, since CSF WBCs and/or protein may be elevated in the natural history of HIV infection and other diseases.

The FTA-Abs CSF test is also available for testing CSF specimens, though it lacks the specificity of the VDRL-CSF test. A nonreactive result, however, carries a high negative predictive value and is recommended by some experts to exclude neurosyphilis in VDRL-CSF-negative cases in which minimal abnormalities in WBCs and/or protein raise suspicion for active neurosyphilis.

Improved methods for diagnosing neurosyphilis are needed. Use of PCR for identification of *TP* in CSF has been disappointing, due to inadequate sensitivity and the inability to distinguish between pathogenic and nonviable treponemes.

**New rapid tests for syphilis.** Given multiple incentives to accurately diagnose syphilis at the point of care, including the need to identify those infected and initiate treatment of the index case and partners early, numerous rapid treponemal tests have been developed for field-based use. None of these is yet cleared for use in the United States. A recent review of six such tests by the World Health Organization demonstrated a range of sensitivities from 84.5% to 97.7% and specificities from 92.8% to 98.0%.<sup>15</sup> A recent evaluation of three rapid tests by the San Francisco Department of Public Health showed that the Abbott Determine Syphilis TP test (Abbott Laboratories, South Pasadena, CA) had the highest sensitivity, 88% (95% CI 0.81 to 0.96), using whole-blood venipuncture samples, with both 100% sensitivity and specificity on 99 whole-blood fingerstick specimens. The test typically takes 15 minutes to perform, does not require sophisticated laboratory equipment, and costs approximately \$2 per test.

**Table 1: Performance of standard serologic tests for syphilis\***

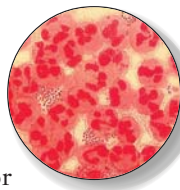
	Stage of untreated syphilis % sensitivity (range)			Specificity % (range)
	Primary	Secondary	Latent	
<b>Nonreponemal test</b>				Nonsyphilis
RPR card	86 (77-99)	100	98 (95-100)	98 (93-99)
VDRL slide	78 (74-87)	100	96 (88-100)	98 (96-99)
<b>Nonreponemal test</b>				
FTA-Abs	84 (70-100)	100	100	87 (84-100)
TPPA **	90-100		50-100	

\* Modified from Larsen, S.A., V. Pope, R.E. Johnson, et al., *A Manual of tests for syphilis*. 9th ed. 1999, Washington, DC: American Public Health Association, except as indicated. Indicated percentage ranges based on CDC studies. (Citation 8 in references.)  
 \*\* Fujirebio Diagnostics Inc., Serodia – TP-PA [package insert, p.4]. Malvern, PA, 2002, comparing TPPA with MHA-TP in population of patients suspected of syphilis by symptoms and history, as well as population of normal controls. (Not cited in references.)

**Neisseria gonorrhoeae**

*Neisseria gonorrhoeae* (*NG*) is a Gram-negative diplococcus that commonly infects the mucosa of the urethra, cervix, rectum, and throat. It frequently presents as an uncomplicated, symptomatic infection at one or more of these sites. In women, untreated lower genital tract infection, which more often may be asymptomatic, may progress to pelvic inflammatory disease (PID). Repeated cases of PID increase the risk for chronic pelvic pain, ectopic pregnancy, and infertility.

The mainstays of *NG* detection have been direct visualization by Gram-stain using light microscopy, as well as bac-



terial culture. In recent years, testing for *NG* has been reshaped by the emergence of widely available nucleic acid amplification tests (NAATs), often supplanting the need for invasive and uncomfortable collection of genital specimens. Nucleic acid probe hybridization assays remain available. Serologic tests for *NG* are not available, and although enzyme immunoassay (EIA) tests are available, their test performance is inferior to standard methods. A 20-minute rapid assay, based on an optical immunoassay (OIA) technology (Thermo Electron Corp., Waltham, MA), was recently cleared by the Food and Drug Administration (FDA) for testing of endocervical and male urine specimens in symptomatic persons. Its role in clinical care remains to be determined.

**Gram stain and culture for NG.** Gram staining of clinical specimens allows direct visualization of Gram-negative diplococci within polymorphonuclear leukocytes, supporting the diagnosis of gonorrhea. In symptomatic male urethritis, the sensitivity and specificity of Gram stain approximate that of culture; the utility of Gram stain in asymptomatic urethritis is not as well validated. Gram staining of endocervical smears should not be undertaken unless a skilled microscopist is available. Since saprophytic nonpathogenic *Neisseria* species colonize the pharynx and rectum, Gram staining of such specimens may lead to false-positive results and should not be done routinely.<sup>16</sup>

Bacterial culture is the gold standard for diagnosis of gonorrhea. Growth of *NG* is selected using Thayer-Martin medium and 5% CO<sub>2</sub> incubation at 35°C to 36.5°C. Plates are examined at 24-hour intervals through 72 hours' incubation. A candle-extinction jar may be used at field sites where CO<sub>2</sub> incubation is unavailable. Self-contained transport systems, such as the GasPak CO<sub>2</sub> iPouch (BD, Franklin Lakes, NJ), are available to substitute for incubation. Presumptive diagnosis of culture isolates requires a Gram stain and oxidase test. Carbohydrate-degradation tests are used to differentiate non-*NG* species and related organisms. *NG*-specific tests, such as the nucleic acid probe test may be used to confirm isolates. In general, the sensitivity of culture is affected by a host of factors including specimen-collection technique, condition of medium prior to use, and handling issues (e.g., timing of plate inoculation, transit time, and storage of plates).<sup>16</sup>

**Nucleic acid amplification testing for NG.** Available nucleic acid tests are based on probe hybridization technology, as well as amplification methods. Two such hybridization assays are FDA cleared, including the PACE 2 (Gen-Probe) and Hybrid Capture II (Digene Corp., Gaithersburg, MD) tests, both of which detect *NG* and *C. trachomatis* in the same specimen. A version of these two tests identifies the presence of either bacterium and, when positive, must be followed up by the more specific test. A particular advantage of these tests is the ability to store and transport specimens for one week. Sensitivity of these tests is somewhat lower than that of NAATs.

Diagnosis of gonorrhea has undergone dramatic change with the advent and widespread use of *NG*-specific NAATs for urine, urethral, and endocervical specimens, first available in 1993. While different amplification techniques and nucleic acid targets underlie the variety of available NAATs, from a practical clinical perspective, the noninvasiveness and portability of test collection has increased the potential for more widespread screening. Furthermore, features translating into enhanced clinical practice and public health efforts include: comparable sensitivity to reference-standard tests, such as culture; noninvasiveness of specimen collection; ease of transport; the accurate performance of these tests with a

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variety of specimen sources; and the ability to detect nonviable organism.<sup>17</sup>

Use of nucleic acid amplification technology for testing of specimens from the oropharynx, rectum, and vagina is subject to limited FDA clearance. In January 2004, the FDA cleared the Gen-Probe APTIMA COMBO 2 for testing self-collected vaginal swabs. While research studies have validated the use of NAATs in certain anatomic sites<sup>18-20</sup>, compliance with the Clinical Laboratory Improvement Amendments (CLIA) of 1988 requires clinical laboratories to conduct internal evaluations prior to testing specimens using collection methods not cleared by the FDA. Also, the assays are subject to factors that inhibit enzymatic amplification, which may be associated with false-negative results, although internal control procedures instituted by manufacturers have minimized this problem. Finally, a predictable rate of false-positive results using NAATs, as has been observed, may appreciably lower the positive predictive value of these tests when employed in populations with a low-prevalence of NG.<sup>21</sup>

The availability of such technology also allows testing of pooled patient specimens. Pooling permits substantial cost savings of reagents and technicians' time, especially when testing in low-prevalence populations. Numerous published studies have demonstrated pooling does not significantly compromise sensitivity or specificity.<sup>21-23</sup> Pooled testing, however, is not cleared by the FDA. Local laboratories must perform validation studies to comply with regulations under CLIA. Turn-around time is increased if positive specimens are not retested the same day, and care in specimen processing is required to avoid laboratory error. Laboratories planning to implement pooling protocols should review CDC recommendations.<sup>16</sup>

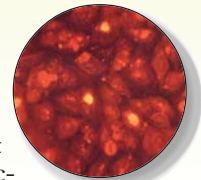
Available NAATs include: the PCR-based Roche AMPLICOR; the BDProbeTec ET, using strand displacement amplification; and the Gen-Probe APTIMA and APTIMA COMBO 2, using transcription-mediated amplification of ribosomal RNA targets. Institutional and laboratory selection

among candidate assays requires a review of clinical service goals, population characteristics, and laboratory issues — such as physical space constraints, including segregation of sample processing and amplification areas, technician resources, and other economic and logistical factors.

Despite the advantages of NAATs for NG, a significant benefit of culture is the capacity to determine antibiotic susceptibility of derived isolates. Such monitoring has become increasingly important given the recent emergence of drug-resistant gonococcal species, such as fluoroquinolone-resistant NG.<sup>25</sup>

**Chlamydia trachomatis**

Infection with *Chlamydia trachomatis* (CT) is the most commonly reported STD in the United States. It is an obligate intracellular bacterium with a complex life cycle that infects mucosa of the lower genital tract, rectum, and throat. It is also the cause of trachoma and lymphogranuloma venereum, rarely seen in this country. Most infection with CT is asymptomatic, more so in women, in whom untreated infection can lead to PID, ectopic pregnancy, and infertility.



Testing for CT has undergone dramatic change with the widespread use of NAATs. Compared to NG testing, alternatives to nucleic acid tests for CT diagnosis are more numerous and include cell culture, EIA- and DFA-based tests, and rapid tests. Serologic testing for acute CT is neither sensitive nor specific and should not be used. Despite a broad array of test alternatives, as well as statistical challenges in comparing accuracy of competing modalities, growing evidence supports use of NAATs for routine CT diagnosis, utilizing a variety of specimen types.

**Cell/tissue culture for CT.** A variety of methods is available to culture CT directly. Usually, a susceptible cell line is inoculated by a patient specimen, and within 72 hours of incubation, characteristic inclusions of CT elementary and reticulate bodies can be visualized using a CT-specific fluorescein-conjugated antibody. The process is labor intensive, costly, and subject to diminished sensitivity if specimens are handled improperly. Given interlaboratory variation in culture routines, performance of this method is variable. Since the advent of noninvasive nucleic acid tests, the role of CT culture has diminished. Yet given its absolute specificity, in most situations, culture remains the test of choice for legal purposes in sexual abuse cases.

**Nucleic acid amplification testing for CT.** NAATs available for CT screening include the Roche AMPLICOR, BDProbeTec ET, and Gen-Probe APTIMA assays. A particular advantage of these tests is the ability to test urine samples, in addition to swabs of the endocervix and urethra. Since the sensitivity of these tests exceeds culture, the precise estimation of sensitivity and specificity is difficult and depends on the test used and the specimen tested, though most experts agree that sensitivity of NAATs exceeds that of other tests, especially rapid tests.<sup>16</sup> Use of NAATs identify up to 30% of CT infections that would be missed by other methods.<sup>25</sup> As mentioned previously, in January 2004, the FDA cleared the Gen-Probe APTIMA COMBO 2 for testing self-collected vaginal swab for CT.

**Other testing methods.** Nucleic acid tests utilizing probe hybridization technology also are available. As discussed earlier, these tests facilitate testing for both CT and GC, simultaneously, are automated and cheap, and do not require refrig-

**Table 2: Performance of selected tests for *Neisseria gonorrhoeae*\***

	Sensitivity (%; range)	Specificity (%; range)
Culture	85-95	100
Nucleic acid amplification test †		
Urine specimens, LCR	94.3-100	98.2-100
Nucleic acid hybridization (nonamplified) test †		
Endocervical swab specimens	85.4-100	93.5-99.6
Male urethral swab specimens	91.5-100	82.2-98.9
<b>Performance of selected tests for <i>Chlamydia trachomatis</i>**</b>		
Cell culture (male and female)	30-86	100
Nucleic acid amplification test †		
Endocervical specimens		
PCR	85.0-100	99.0-100
SDA	92.8-100	98.2-99.3
TMA	82.5-100	99.4-100
Urine specimens		
PCR	86.7-100	97.0-100
SDA	92.8-100	93.8-99.3
TMA	82.5-100	98.7-100
Nucleic acid hybridization (nonamplified) test †		
Male and female swab specimens	75.3-91.7	99.0-100

\* Koumans EH, Johnson RE, Knapp JS, St Louis ME. Laboratory testing for *Neisseria gonorrhoeae* by recently introduced nonculture tests: a performance review with clinical and public health considerations. Clin Infect Dis. 1998 Nov; 27(5):1171-1180. (Not cited in references.)  
 \*\* Evaluation of Diagnostic Tests for Detection of Genitourinary Chlamydia Infections, 2001. California Department of Health Services STD Control Branch and the Department of Epidemiology and Biostatistics: Berkeley, CA. ([http://www.ucsf.edu/castd/std\\_publications.html](http://www.ucsf.edu/castd/std_publications.html))  
 † Gen-Probe PACE 2, compared to culture as reference standard.  
 ‡ NAATs include polymerase chain reaction (PCR, e.g., Roche AMPLICOR), strand displacement amplification (SDA, e.g., BDProbeTec), and transcription-mediated amplification (TMA, e.g., Gen-Probe APTIMA) tests; ligase chain reaction (LCR, e.g., Abbott LCx NG Assay) no longer commercially available in the U.S.

eration of transported specimen containers. They cannot be used, however, to test urine and are not as sensitive as NAATs. EIA- and DFA-based tests for *CT* antigen detection have similar disadvantages and are more technically difficult to perform.<sup>27</sup>

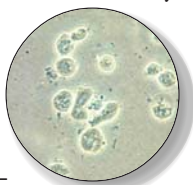
**Rapid point-of-care tests for *CT*.** Rapid point-of-care tests for *CT* include QuickVue Chlamydia (Quidel Corp., San Diego, CA), Clearview Chlamydia (Unipath Limited, Bedford, U.K.), Biostar Chlamydia OIA (Thermo Electron Corp.), and Abbott Testpack (Abbott Laboratories, Abbott Park, IL). These tests offer rapid results, but entail higher cost and decreased sensitivity compared to other testing modalities, such as NAATs. Reliance on these rapid tests may slow adoption of NAATs, especially given logistical and practical considerations in some laboratories, such as lack of staff and loss of revenue from samples redirected to larger labs. In Seattle, for instance, a survey of laboratory directors revealed continued reliance on lower-sensitivity tests for *CT* six years after the advent of NAATs.<sup>28</sup> While use of rapid tests for *CT* may be justified in settings where follow-up is extremely unlikely, their utility must be weighed against the benefit of more accurate, less costly, laboratory-based NAATs now in wide use.

### **Trichomonas vaginalis**

The most prevalent nonviral sexually transmitted infection worldwide, trichomoniasis is caused by the protozoan *Trichomonas vaginalis* (*TV*). Infection is often asymptomatic, especially in men. In women, trichomoniasis may cause a malodorous yellow-green discharge, along with vulvo-vaginal irritation. Since infection is often asymptomatic or mild in men, affected women are often re-exposed to *TV* from untreated male partners. Trichomoniasis is a risk marker for other STDs, inflammation from the infection may potentiate HIV transmission, and infection during pregnancy may contribute to poor birth outcomes.<sup>3,4</sup>

**Wet mount and culture for *TV*.** Using light microscopy, examination of saline wet-mount specimens obtained from pelvic examinations allows direct visualization of motile trichomonads, though sensitivity is operator-dependent and rarely exceeds 70%.<sup>6</sup> Sensitivity of *TV* detection is increased by culture under microaerophilic conditions. A variety of culture media is commercially available for diagnosis of *TV*. Also available is the InPouch *TV* Culture System (Biomed Inc., San Jose, CA). The test involves simultaneous performance of wet mount and can detect presence of a single organism. Viable organisms are required, and inoculated pouches must be transported to the lab within 48 hours, incubated at 37°C, and read over five to seven days.

**Additional tests for *TV*.** A variety of immunoassays, latex agglutination assays, and nucleic acid-based tests have been developed for use on serum and genital samples. Recently, the Xenostrip *TV* Trichomonas Test (Xenotope Diagnostics Inc., San Antonio, TX), a qualitative immunochromatographic assay, was FDA cleared for use on clinician-collected vaginal swab specimens.<sup>29</sup> The test provides results within 10 minutes and has a relative sensitivity compared to culture of 99% to 100% and specificity of > 98%. A PCR-based test has not been cleared for use in the United States, though local laboratories may develop and evaluate such assays. A recent evaluation of five PCR primers using self-collected vaginal swabs, with comparison to culture and/or EIA, demonstrated only 60% to 90% sensitivity of PCR.<sup>30</sup> Prior investigators reported sensitivity and specificity of *TV* PCR



using vaginal swabs of 97 and 98%, respectively, compared to sensitivity of culture and wet prep of 70 and 36%.<sup>31</sup>

### **Herpes simplex virus**

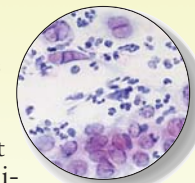
Infection with herpes simplex virus is among the most common STDs in the world, affecting more than one in five adults in the United States alone.<sup>32</sup> Most genital herpes is caused by HSV type 2. Initial, or primary, episodes may cause severely painful vesicles and ulcers, with or without lymph-node tenderness and enlargement. Less often, primary herpes involves systemic symptoms and causes "aseptic" meningitis. A chronic state of infection then ensues, in which recurrent symptoms vary in tempo and severity. Irrespective of symptoms, intermittent shedding of virus is ongoing, as is the risk of transmitting the virus to sex partners.<sup>33</sup> The infection cannot be cured.

**The role of type-specific serologic testing for HSV.** Clinical diagnosis of genital HSV is insensitive and nonspecific.<sup>34</sup> The diagnosis of HSV-2 infection has been aided by the availability of serologic tests for type-specific antibody to the virus, supplanting crude antigen testing. Use of these tests necessitates appropriate patient-centered counseling about transmission risk, prevention steps, and treatment, especially since herpes infection continues to carry significant stigma in the general population. The tests cannot differentiate recent infection from that acquired remotely. In many instances, patients diagnosed with HSV-2 can recall symptoms consistent with the infection. Diagnosis of HSV infection may facilitate interventions aimed at minimizing secondary transmission. For instance, a recent clinical trial showed daily antiviral therapy reduced the risk of HSV-2 transmission to uninfected partners.<sup>35</sup>

In general, serologic tests for HSV-2 should be available to those who request them, especially those who are partners to HSV-2-infected persons. Most authorities, however, recommend use of such tests for screening only in high-risk populations, such as persons with other STDs and those with or at risk for HIV infection. Screening the general population, including pregnant women, is not recommended.<sup>33</sup> In particular, serologic tests may be most useful for diagnosing genital herpes on the first presentation of genital symptoms when culture and antigen detection tests are not available, when a recurrent lesion is repeatedly culture-negative, or when a patient's history of symptoms is consistent with genital herpes. A "window period" of six to 12 weeks, during which time antibodies have yet to form after primary infection, however, complicates the use of HSV serology in these instances.<sup>32</sup>

**Type-specific antibody tests.** The Western blot assay is the gold standard technique for HSV antibody detection and can differentiate between type 1 and 2 antibodies. Limitations include high cost and labor intensity, such that tests have not been developed commercially.<sup>36</sup> Type-specific protein glycoprotein G2 antibody tests for HSV-2 include the HerpeSelect-2 ELISA IgG and HerpeSelect 2 Immunoblot IgG tests (Focus Technologies Inc., Herndon, VA). Sensitivities of these tests vary from 80% to 98%, with specificities  $\geq 96\%$ .<sup>6,35</sup> Since this is a serologic assay measuring IgG, the sensitivity relies on the time since infection, with median positivity occurring within two weeks. Rarely, HSV-2 can be gG2 deficient, resulting in a false-negative result.

**Detection of HSV in genital ulcer material.** In addition to serologic testing, direct laboratory evaluation of suspicious



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genital lesions remains important to clinical management of genital ulcer disease. In the United States, HSV and *TP* cause most such ulcers. While direct laboratory evaluation of genital lesions to rule in syphilis is often most pressing, depending on clinical circumstances lesions suspicious for HSV should be evaluated by collecting a specimen for culture or DFA. Cell culture is the preferred test for laboratory evaluation of suspicious mucocutaneous lesions. The sensitivity of culture is higher for primary vs. recurrent lesions and declines as lesions heal, often several days after onset. HSV culture requires live virus, infected cells, special care in sampling and transport to a virology laboratory; it is time-consuming and expensive. Of note, the enzyme-linked virus-inducible ELVIS HSV system is a 24-hour culture system (Diagnostic Hybrids Inc., Athens, OH) that is commonly used but less sensitive than traditional culture.

Other techniques for direct examination are available. DFA tests, such as the Light Diagnostics HSV 1/2 Typing DFA Kit (Chemicon International Inc., Temecula, CA) and MicroTrak DFA test (Wampole Laboratories, Princeton, NJ), approximate the sensitivity of culture and can distinguish HSV-2 from HSV-1. Compared to other available techniques, cytologic detection of HSV infection, such as by Tzanck prep, is insensitive and nonspecific and thus not recommended. PCR assays for HSV, including multiplex assays, are highly sensitive but are not FDA cleared and thus not readily available. If culture and antigen detection tests are not available or if a recurrent lesion is repeatedly culture-negative, a serologic test for HSV-2 should be considered.

## Conclusion

The laboratory plays a crucial role in providing the tools necessary to effectively diagnose and control the spread of sexually transmitted infections. Numerous well-established assays continue to be used in the diagnosis of some STDs. Newer tests, however — some based on advances in molecular diagnostics — have radically improved the efficiency and accuracy of STD diagnosis. Rapid tests, point-of-care testing, and nucleic acid amplification technology represent advances addressing long-standing barriers in STD diagnosis, while meeting with patient acceptance given noninvasiveness, ease of collection, and quick turnaround of many such tests. By working closely with laboratorians, clinicians and public health officials are realizing new opportunities for the early recognition, treatment, and control of STDs. □

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# Real-time STD reporting is just around the corner!

By John Fisher

The Center for Disease Control and Prevention (CDC) has a goal of receiving all legally reportable laboratory results from all agencies in real time. Real-time transfer of results will need to come from the testing agency, through the state departments of health to the CDC in a matter of minutes — not days. This method has shown an increase in the reporting rate. The electronic format the CDC has selected for the transfer of this information is HL7. This information will include complete patient demographics, testing location, SNOMED (systematized nomenclature of medicine) codes, and LOINC (logical observation identifiers names and codes). As this standard is adopted by local and state agencies, they will require the testing labs to adopt these standards, too.

Is your laboratory information system (LIS) ready for real-time tracking and reporting? With interfaces between your analyzers and your LIS, you can identify and track positive samples, and with an interface to the local health department's laboratory information management system (LIMS) or its epidemiology information system, you can make reporting seamless. If you are not sure, or if you are in the market for a new analyzer or lab information system, you may want to consider the following before purchasing.

## Analyzer interface to your LIS

An LIS should interface with the various instruments used for STD testing. Before you invest in a new analyzer, ask the following:

Can the new analyzer be interfaced to your LIS? If the answer is a resounding "Yes," then you may continue to consider this manufacturer's instrument. If the answer is anything but a firm "Yes," you may want to look at other analyzers.

Assuming you got a strong affirmative, determine the type of interface the manufacturer has developed for the instrument. Most likely, it will be one of two basic types: unidirectional or bidirectional. A unidirectional interface will send results from the analyzer to the LIS; however, orders and patient demographics must be directly entered into the analyzer, as no data is coming from the LIS to the instrument. A bidirectional interface allows the analyzer and the LIS to transmit data back and forth. If you are already entering orders in your LIS, a bidirectional interface with your analyzer eliminates the need for re-entering orders and demographics in the analyzer.

## LIS interface to your instrument

After you have determined the type of interface for your analyzer, your focus should shift to your LIS. Confirm that your

LIS vendor can develop an interface to meet the manufacturer's specifications, and find out the time frame and what costs will be incurred.

Next, determine how the interface will affect the current workflow with your LIS. Does the analyzer automatically send results to the LIS, and will additional manipulation of those results be required by the LIS? This could include adding a formula to convert from one unit to another, or converting a numeric value to a text value. Does the analyzer interface require a release of results individually or a batch release of results? Does your LIS allow for an autoapproval based on criteria that you have predetermined? Criteria should be based on the results from the analyzer, as well as the ordering location, physician, and patient.

## Real-time reporting

After test approval, you will need to deliver the results to your local or state department of health. While most LISs deliver the results to the requesting provider(s) via a printed or faxed report, the Internet, or e-mail, the real issue is how your lab delivers a legally reportable result to the department of health.

Health departments have required the reporting of positive tests for years, and depending on their laboratory information management system or their epidemiology information system, the method of reporting will vary. During the past few years, the CDC has tried to define the information it expects to receive from the state departments of health. This will trickle down to local health departments and to those labs performing the testing.

### *Is your laboratory information system (LIS) ready for real-time tracking and reporting?*

Most labs today are faxing or mailing reportable lab results to their health department, but this is quickly changing. With these changes, can your LIS handle the new requirements? Can your LIS recognize a "positive" reportable result and deliver it to the appropriate agency? What method is your LIS using to deliver this information? Are you ready for real-time reporting?

If your lab is testing for legally reportable results, begin thinking about your lab's ability to generate reports in real time. Keep these issues in mind, especially if you are looking at new analyzers or information systems. The key to real time reporting is successfully interfacing your instruments to your LIS, and instantly transmitting these results to your local department of health. □

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