

Current and Best Practices for Testing of Sexually Transmitted Diseases

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Effective diagnosis and treatment of infected persons is a critical component of sexually transmitted diseases (STDs) prevention and control. STD diagnostic methods that are sensitive, rapid and inexpensive are ideal for testing of individuals presenting with genitourinary symptoms and screening of asymptomatic individuals in high risk populations. Fortunately, recent advances in STD diagnostics have provided nucleic acid amplification tests (NAATs) with increased sensitivity compared to older tests and rapid “point-of-care” (POC) tests, with quick turn-around time for results. However, despite their ability to improve best practices for STD management, NAATs and POC tests have not been widely adopted into public and privately funded practices. This may in part be due to barriers ranging from the cost and complexity of some of these methods to unfamiliarity with application of these new technologies for the testing and screening of at-risk patients.

Although federal resources for the control and prevention of STDs are primarily distributed to state and local health departments, the National Health and Social Life Survey reported that STDs are frequently treated in private practice settings.¹ Therefore, consideration of the ideal STD testing methods to use in the apparently low prevalence populations evaluated in private clinics is as crucial as application of these technologies in high prevalence populations presenting to STD clinics and emergency rooms.

New STD Diagnostic Methods

Currently available NAATs for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are based on new technologies, including polymerase chain reaction (PCR), DNA strand displacement assays (SDA), or transcription-mediated amplification (TMA) using vaginal, cervical, urethral or first-void urine specimens (Table 1).² Self-collected vaginal swabs may also be used in settings where

physical examinations are limited for women.³

Diagnosis of gonorrhea and chlamydia was previously reliant on cultures, enzyme immune assays (EIA), and non-amplification DNA probe assays to detect these organisms. The use of NAATs is now considered the “gold standard” for *C. trachomatis*; however, despite the availability of NAATs for gonorrhea, culture remains in widespread use since it allows assessment of antimicrobial susceptibility which is currently unavailable with amplification tests. Combination NAATs

are available for *N. gonorrhoeae* and *C. trachomatis* using a single patient specimen,⁴ which is advantageous among high-risk populations in light of the frequency of co-infections with these pathogens. However, NAATs are not approved for detection of gonorrhea or chlamydia in rectal or pharyngeal specimens.

NAATs also hold promise in the future for diagnosis of other STDs. PCR and TMA for *Trichomonas vaginalis* are more sensitive than culture for diagnosis of trichomoniasis in

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Table 1.
Common STD Syndromes, Most Likely STD Etiologies and Performance Estimates of Selected Available Diagnostic Technologies ^{2,4,7,10-13}

STD Syndrome	Most Likely STD Etiologies	Available Diagnostic Technologies	Estimated Sensitivity (%): Specific (%)*
Vaginitis/ cervicitis	Trichomonas vaginalis	Wet mount microscopy	30-60; 100
		Culture	80-90; 100
		Rapid antigen test	78-83; 98-99
	Chlamydia trachomatis	Enzyme immunoassay	53-76; 95
		Direct fluorescent antibody	80-85; >99
		Probe hybridization	65-83; 99
		Polymerase chain reaction (cervical)	90; 99
		Strand displacement assay (cervical)	93; 98
		Transcription-mediated amplification (cervical)	94; 98
	Neisseria gonorrhoeae	Culture (Thayer-Martin)	80-95; 100
		Probe hybridization	93-96; 99
		Polymerase chain reaction (cervical) (urine)	92; 100 65; 100
		Strand displacement assay (cervical) (urine)	97; 98 -100 85; 99-100
		Transcription-mediated amplification (cervical) (urine)	99; 99 91; 99
Urethritis (males)	Chlamydia trachomatis	Enzyme immunoassay Direct fluorescent antibody Probe hybridization	As above
		Polymerase chain reaction (urethral) (urine)	96; 99 90; 98
		Strand displacement assay (urethral) (urine)	95; 94 93; 94
		Transcription-mediated amplification (urethral) (urine)	95; 98 97; 99
	Neisseria gonorrhoeae	Culture Probe hybridization	As above
		Polymerase chain reaction (urethral) (urine)	97; 97 94; 100
		Strand displacement assay (urethral) (urine)	99; 92-100 98; 93-100
		Transcription-mediated amplification (urethral) (urine)	99; 98 97; 100
	Trichomonas vaginalis	Culture	50; 100
Genital ulcers	Herpes simplex virus	Culture	73-100; 100
		Type-specific ELISA serology	81-100; 96-99
		Rapid antibody test (HSV-2)	93-96; 95-98
		Polymerase chain reaction	98-100; 98-100
	Treponema pallidum	Non-treponemal tests	78-86; 98
		Treponemal tests	87-100;
		Rapid antigen tests	70-88; 100
		Enzyme immune assay	71; 98

* Ranges for test performance of the diagnostic technologies reflect broad differences in reference standards and patient populations.

both men and women, but these molecular tests are available only in clinical research settings.⁵ PCR and TMA assays have been developed for *Mycoplasma genitalium*, which has been recently established as a case of non-gonococcal urethritis (NGU) in men, but the future clinical application of these tests is uncertain.⁶ For the evaluation of genital ulcer disease, a PCR for herpes simplex virus (HSV) has enhanced performance over viral isolation with culture, but is not yet approved by the Food and Drug Administration (FDA) for testing of genital specimens.⁷ A multiplex PCR test for HSV, *Treponema pallidum* (syphilis), and *Haemophilus ducreyi* (chancroid) has been developed and continues to be investigated for clinical application.⁸

For human papilloma virus (HPV), there is a commercially available signal-amplified nucleic acid assay and PCR to detect high-risk HPV types associated with cervical cancer.⁹ These HPV DNA tests are currently being considered for cervical cancer prevention (along with the Pap smear) including primary screening for high-grade lesions among women >30 years of age. They are not intended to assist in the diagnosis of external genital warts which are primarily associated with benign HPV types.

There are several STD rapid tests which have the advantage of immediate diagnosis in clinics serving both high- and low-prevalence populations. For *T. vaginalis*, a POC test that uses an immunochromatographic capillary flow (dipstick) assay to detect parasite antigens in vaginal specimens can provide results in 10 minutes.¹⁰ Compared to culture, this rapid test for trichomoniasis has a sensitivity and specificity which is superior to wet mount microscopy (Table 1), but is only approved for use in women. Rapid syphilis tests have also been introduced using an immunochromatographic strip with finger-stick or whole blood to detect antibodies to *T. pallidum* antigens.¹¹ However, these tests would be reactive in all patients with prior syphilis, which limits their potential use for testing or screening in low prevalence clinic populations.

There are several new serologic tests for diagnosis of syphilis and HSV that are worth noting. Recently, an immunoglobulin G (IgG) EIA has become available as a diagnostic test for syphilis.¹² This test, which detects treponema-specific antibodies, has the potential to be automated and produces an objective result without requiring confirmation (unlike the reactive plasma reagin [RPR] or other non-treponemal tests). Clinicians now have a choice of several type-specific HSV antibody tests based on the glycoprotein G antigens of HSV-1 and HSV-2.¹³ These serologic tests in an enzyme-linked immunosorbent assay (ELISA) format can be used to supplement culture and physical examinations to confirm a clinical diagnosis, to diagnose a person with unrecognized infection, and to manage sexual partners of persons with genital herpes. In addition, rapid POC tests for HSV-2 antibodies (not HSV-1) using capillary blood or serum are also available and can provide results within six minutes.

Current Practices for STD Testing

Unfortunately, publicly-funded STD clinics in North Carolina that evaluate large numbers of patients with suspected

STDs have limited access to these advanced technologies for routine STD testing and screening. Currently, evaluation of vaginitis still relies on insensitive wet mount microscopy for diagnosis of candidiasis, bacterial vaginosis, and trichomoniasis. While a urethral Gram stain is typically performed in the evaluation of NGU, pathogens other than *C. trachomatis*, including *T. vaginalis* and *M. genitalium*, are not usually tested for in men. Testing for gonorrhea and chlamydia still rely on culture or DNA hybridization probes in many public clinics. Targeted screening for chlamydia is frequently performed based on age or gender due to limited funds. Initial testing for syphilis consists of the non-treponemal tests, which require confirmation at the state laboratory (which recently switched to the IgG EIA for *T. pallidum* for confirmatory testing). Some local public health department laboratories have the capability to perform stat RPRs with available results in 15 minutes and darkfield microscopy for direct visualization of treponemes. Diagnosis of HSV or HPV is often based on clinical presentations, since HSV viral cultures that can be sent to the state laboratory are usually reserved for atypical lesions.

Emergency rooms and private clinics may be better able to use newer technologies for detecting bacterial STDs, including gonorrhea, chlamydia, and syphilis. However, a national survey of physicians conducted in 2002 found that only 1.3% and 1.8% of clinicians used newer urine-based tests for gonorrhea and chlamydia, respectively, although these tests are less invasive and more acceptable to patients.¹⁴ In general, there is little to no information in the literature on the use of newer diagnostic methods for the other bacterial and viral STDs in these clinical settings.

Although some emergency rooms that serve a large number of high-risk patients in urban areas have been reported to have a high prevalence of STDs like gonorrhea ranging from 1.7-11.0%,¹⁵ screening rates in these settings are typically lower than in other settings. A recent national survey of physicians, in which the majority practice in private clinics, reported that fewer than one-third routinely screened men or women (pregnant or non-pregnant) for STDs.¹⁴ The survey found that screening rates among non-pregnant women ranged from 20-35% and were slightly higher for pregnant women at 30-32%. Physicians in the survey who saw male patients rarely screened for syphilis, gonorrhea or chlamydia.

Opportunities for Best Practices

With the increasing availability of improved technologies for STD diagnosis, including highly sensitive and rapid tests, we now have the opportunity to improve current practices for STD testing and screening in various clinical practices as well as non-traditional settings. However, the costs and complexities of the NAATs for gonorrhea and chlamydia are barriers to their widespread application, especially in STD clinics with limited funding or small private practices without access to advanced laboratories. Although they are less sensitive than NAATs, POC tests may be more affordable and easier to implement. Positive results from these tests eliminate the need for a return

visit and increase treatment rates; therefore, POC tests should be strongly considered, especially in the diagnosis of *T. vaginalis* and HSV-2 infections which are the most common STDs.

Public STD clinics clearly need access to sensitive molecular diagnostic tests for gonorrhea and chlamydia among all high-risk patients, including men. However, *N. gonorrhoeae* cultures would still be useful in cases of persistent infections in order to test for antimicrobial resistance. *T. vaginalis* testing should become routine in both men and women using culture or rapid antigen tests; wet mount microscopy alone is clearly suboptimal for organism detection. Use of the HSV type-specific serology and the POC test for HSV-2 could greatly enhance the diagnosis and management of patients with atypical lesions as well as their sexual partners. The availability of the multiplex PCR test for detection of *T. pallidum*, HSV, and *H. ducreyi* would assist in the differential diagnosis of genital ulcers which are frequently seen in these clinics.

STD management in emergency rooms and private clinics could be optimized with the use of urine-based NAATs for *N. gonorrhoeae* and *C. trachomatis* and POC tests for *T. vaginalis* and *T. pallidum* for testing of symptomatic persons. Utilization of POC tests that can be performed in less than 30 minutes in the emergency room or a doctor's office would allow rapid diagnosis and treatment without a need for subsequent follow-up appointments.

Emergency rooms and private practices present an opportunity for targeted STD screening among asymptomatic at-risk patients, who can be easily determined by adding a brief sexual

history as part of their clinic encounters. Adolescents, substance abusers, and persons who report multiple sexual partners should be strongly considered for screening, at least for the bacterial STDs using non-invasive molecular assays or POC tests. The positive predictive values of NAATs, like that of all diagnostic tests, is reduced in low prevalence populations with a greater potential for false-positive tests. However, their increased sensitivity compared to culture make NAATs particularly useful in detecting asymptomatic infections among persons with lower organism burdens. Although STD screening of sexually active women should be prioritized, screening of high-risk asymptomatic men for the most common STDs is important to prevent transmission of undetected infections to their sexual partners.

Approximately 1.3 million cases of bacterial STDs (gonorrhea, chlamydia, early syphilis) were reported in the United States in 2004, and roughly 47,000 cases were reported in North Carolina in 2005.¹⁶⁻¹⁷ In 1994, the total costs for the most common STDs and their sequelae, including pelvic inflammatory disease and adverse pregnancy outcomes, were estimated to be approximately \$10 billion dollars annually.¹⁸ The epidemiologic and biologic associations between STDs and HIV infection underscore the importance of STD management as an HIV preventive strategy. We need to prioritize funds and efforts towards increased utilization of the newer STD diagnostic tests in public and privately-funded practices to maximize the detection, treatment and prevention of these highly prevalent infections and their subsequent complications. **NCMedJ**

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