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MINIREVIEW

Clinical and Laboratory Testing for *Trichomonas vaginalis* Infection

Barbara Van Der Pol

Division of Infectious Diseases, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, USA

***Trichomonas vaginalis* infection is highly prevalent in the United States and worldwide. Traditional clinical diagnostic methods fail to identify more than half of these infections that, if left untreated, can result in adverse pregnancy outcomes and an exacerbated risk of both acquisition and transmission of HIV. Women bear a disproportionate amount of the burden of these infections, and testing among populations at risk for this disease should be provided. Molecular technologies have expanded our capacity for laboratory-based detection of infection and can be used on samples already being collected for chlamydia/gonorrhea screening.**

Trichomonas vaginalis is the causative agent of the most common nonviral sexually transmitted infection (STI) worldwide. However, our understanding of the epidemiology of trichomonas infection contains substantial gaps since this infection is often not screened for in large, population-based studies and the disease is not well monitored by public health agencies. The prevalence of *Trichomonas* infection is best documented in the United States, where the rates are consistently higher than those of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections combined. In studies representative of the general population, the rates of trichomonas, chlamydia, and gonorrhea infections have been estimated at 3.2, 2.2, and 0.2%, respectively (1, 2). Prevalence estimates among women seeking family planning and sexual health care range from 7.5 to 25% (3, 4). Globally, trichomonas infections account for nearly half of all curable STIs. These estimates are population dependent, with very high rates among some groups and almost no disease in others. In Europe, recent population level studies have estimated the prevalence of disease to be <1% in countries with chlamydia rates similar to those in the United States, suggesting a geographic fluctuation in the underlying presence of disease that is not well described or understood (5). In the United States, geographic fluctuation of rates is observed, but the ratio of trichomonas to chlamydia infections remains similar (6).

The age-specific distribution of trichomonas infection is important since it is substantially different from the distribution seen for chlamydial infections. Chlamydia rates peak among young women 19 to 24 years old (7). In contrast, many studies have shown that the prevalence of trichomonas infection is highest among women 40 to 49 years old (6, 8). This differential distribution has prompted discussion regarding the best populations for testing since trichomonas infection prevalence is not highest among adolescents. However, even among adolescent women, the prevalence rates are substantial and bundled chlamydia and trichomonas infection screening of at-risk women should be considered in order to avoid missed opportunities for disease control.

In the United States and Europe, there are no recommendations for routine screening for trichomonas infections among women in the general population (9). Further, although trichomonas is sexually transmitted and thus, by definition, it infects men as well as women, the sex distribution of infection is highly skewed. In a probability sample of urban youth in the United States, the estimated differential in prevalence was roughly 4-fold (10). As a result of this differential and the asymptomatic nature of infection among men, there are no screening recommendations for men. Further, true diagnostic laboratory tests, as opposed to screening in the absence of symptoms, are rarely performed, even for men with nongonococcal urethritis (NGU). Research studies of men with NGU suggest that *T. vaginalis* is responsible for 10 to 12% of these cases of urethritis (11), one of the most common reasons why men <50 years old seek health care. Certain high-risk populations may warrant routine screening, such as women attending STD clinics, women in correctional facilities, women living in high-prevalence regions, men with urethritis, and sexual partners of infected women (4, 5, 12). However, programmatic decisions to offer screening should be based on local epidemiologic data whenever possible. Populations at exacerbated risk of infection with HIV should be considered for trichomonas screening on the basis of strong epidemiologic data linking trichomonas infection to an increased risk of both acquisition and transmission of HIV (13, 14).

CLINICAL MANIFESTATIONS

T. vaginalis is a free-living protozoan that can colonize mucosal epithelial surfaces. Infection may manifest as a fulminant yellow/gray-green discharge and cause vulvar-vaginal itching and odor (15). Upon clinical examination, punctate friability of the cervix (called a strawberry cervix) may be observed. However, it is important to note that estimates of the proportion of infections that exhibit no symptoms range from 40 to 80% (16, 17). Recommended treatment in the United States is a single oral dose of metronidazole (2 g) or tinidazole (2 g) (7). When left untreated, trichomonas infection may be resolved through host immunity (18) or may remain subclinical (19). Little is known about the natural history of infection in either men or women. Infections during pregnancy have been associated with adverse outcomes such as premature rupture of membranes, low birth weight, and preterm delivery. A meta-analysis of randomized clinical studies estimated that women with trichomonas infections were 1.4 (95% confidence interval, 1.15 to 1.75; $P = 0.001$) times more likely to have a preterm birth than women without trichomonas infections (20). Undetected/untreated infections also increase the risk of HIV acquisition and transmission, as mentioned above. Several studies have shown a relationship between trichomonas infection and an increased risk of infection with HIV in regions where HIV is endemic. This epidemiologic association is biologically plausi-

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Address correspondence to bvanderp@uab.edu.

ble since trichomonas infection induces an inflammatory response that recruits HIV-susceptible cells to the site of exposure (the vagina). Further, infection may cause microabrasions of the vagina, which allows HIV access to the bloodstream. Estimates of the risk of HIV acquisition range from 2.1- to 2.8-fold higher for women with trichomonas infections attending family planning clinics in sub-Saharan Africa (13, 14). Of additional concern is the converse relationship that women with HIV are at elevated risk of acquiring trichomonas infection (13). This is problematic since discharge-causing infections are directly related to increased genital compartment HIV loads (21), the single most important factor in HIV sexual transmission. Theoretical estimates based on modeling predict that, among women with HIV, detection and treatment of trichomonas infections would lead to cost savings because of the number of HIV transmissions prevented by this strategy (22, 23). Unfortunately, randomized controlled studies designed to assess the impact of trichomonas infection control as an HIV prevention method are too costly to perform, and thus, we have no evidence regarding the real impact that could be achieved by simple diagnostics and effective treatment of *T. vaginalis* infections.

DIAGNOSTICS

Detection of infection may be accomplished by a variety of techniques, ranging from clinical diagnosis to laboratory-based testing (Table 1). Clinical diagnosis is appropriate for women presenting with symptoms of infection; discharge, itching, and/or vaginal odor. The most commonly used diagnostic test is wet preparation microscopy. A swab is used to collect material from the vaginal fornix and placed into a small amount, 0.5 to 1.0 ml, of normal saline. A drop of the saline preparation is placed on a slide and viewed with a microscope with a 40× objective. Motile trichomonads can be seen moving in the preparation with a distinctive asynchronous motion. Flagella and an undulating membrane on one side of the organism must be visible. Care must be taken to ensure that the organisms are motile because the size of a trichomonad is approximately the same as that of a white blood cell, which will likely be present in specimens from patients with a discharge. Organisms lose motility *ex vivo* because of temperature shock, so slides should be prepared and read as soon as possible following collection in order to avoid false-negative results. While motile trichomonads are very distinctive if observed microscopically, the organism load required in order to capture sufficient organisms for visualization is unknown. Comparison with newer diagnostic assays suggests that wet preparation microscopy is only 40 to 60% sensitive, even among symptomatic women (24). However, observation of motile trichomonads is highly specific and thus is very useful for determining a course of treatment. Microscopy should never be used as a screening method for asymptomatic women.

Better alternatives to microscopy are available that can detect more infections and can be used independently of the presence of symptoms. One such assay that can be used at the point of care (POC) is the OSOM (Sekisui, Framingham, MA) immunochromatographic enzyme assay performed on a capillary flow dipstick device with a vaginal swab specimen. The assay relies on a latex-tagged antibody to bind specifically to trichomonas proteins and a secondary capture antibody to bind the complex to the lateral-flow device. The test is Clinical Laboratory Improvement Amendments (CLIA) waived, making it suitable for POC use, and results are available within 15 min. The performance characteristics of this assay are quite good. In multiple studies, the use of this test has improved case finding significantly compared to the number of cases found by only microscopy. In studies comparing the OSOM assay with nucleic acid amplification tests (NAATs), the POC test performed quite well, with sensitivity similar to that of NAATs (>80% compared to NAATs at >90%) (24, 25). The only drawback to this test is that it can only be used for women and cannot be bundled with POC chlamydia/gonorrhea screening. Thus, women who are treated for trichomonas infection during an office visit based on a positive OSOM test may still need to return for a second visit if they are subsequently diagnosed with either chlamydia or gonorrhea on the basis of a laboratory diagnostic test.

The use of any POC test is clearly warranted when symptomatic women present for diagnostic evaluation. The ability to define the causative agent and provide appropriate treatment while the patient is still in the clinic is critically important to reducing disease transmission (26). However, when relying on microscopy, providers should be aware that negative results may be false, missing 50% or more of infections, and laboratory evaluation can provide useful information. This is particularly relevant for women without signs or symptoms and for testing of men (even those with symptoms). Wet preparation microscopy is a useful first test for women with symptoms since treatment can be provided immediately. However, it should be used in a cascade process where microscopy-negative samples are sent to a laboratory for further evaluation.

Laboratory-based diagnostics have the disadvantage of not providing immediate results while the patient waits so that treatment can be provided before the end of the patient visit. However, the ability to isolate organisms, utilize molecular technologies, test for multiple pathogens that are frequently found together, or run large batch sizes may result in economies of scale and additional medical information that support the use of these tests in many settings. These advantages are particularly relevant for women without symptoms but whose risk of infection is high and for men in general since the organism load appears to be lighter in these groups and thus high sensitivity and low limits of detection are necessary.

The traditional laboratory-based diagnostic test was trichomonas culture with a modified Diamond medium. Cultures are assessed by microscopy of a slide prepared from a drop of the culture medium daily for up to 7 days. A significant advancement in culture was seen with the availability of the InPouch (Biomed Diagnostics, Santa Clara, CA) system, which contains culture medium in a pouch that can be placed on a microscope stage. Thus, the entire volume of the culture can be evaluated for the presence of trichomonads. The InPouch system achieved an incremental increase in sensitivity over routine culture (27), but the true advantage of the system is the improved logistics. This system has potential utility in resource-constrained settings where clinic-based microscopy may not be feasible but a central laboratory may be able to provide low-tech needs such as incubation and microscopic evaluation. In high-resource settings, the only real utility of culture is to obtain viable organisms that may be relevant for antimicrobial susceptibility testing. Reports of resistance to metronidazole, the most commonly used treatment for trichomonas infections, are described in the literature, but the true extent of this problem is unknown and susceptibility testing is not performed routinely (5).

Non-culture-based tests often provide an increase in the ability to detect infections since viable organisms are no longer required. The first commercially available non-culture-based test for *T. vaginalis* was the Affirm VPIII (BD, Sparks, MD). This assay utilizes DNA probe technology to detect *Candida albicans*, *Gardner-*

TABLE 1 Features of diagnostic methods for detection of *T. vaginalis*

Assay	Time to result	Equipment requirements	Sample type(s)	Cotesting option(s)	Relative cost[a]	Relative sensitivity[b]	Comment(s)
Wet preparation microscopy	5 min	Microscope with 40× objective	Clinician-obtained vaginal swabs	Bacterial vaginosis clue cells	$	+	Trichomonads must be motile to avoid confusion with lymphocytes; motility decreases rapidly following sample collection
OSOM	15 min	None	Clinician-obtained vaginal swabs	None	$$	+++	CLIA waived—true POC test
Culture	1–7 days	Incubator, microscope with 40× objective	Clinician-obtained vaginal swabs	None	$$	++	CIA moderate
Affirm VPIII	<1 h	Affirm VPIII instrument	Clinician-obtained vaginal swabs	*Gardnerella vaginalis*, *Candida albicans*	$$	++	Cannot be used for asymptomatic screening; CLIA moderate complexity, DNA probe technology
AmpliVue	<1 h	AmpliVue instrument	Clinician-obtained vaginal swabs	None	$$	++	CLIA moderate complexity, DNA amplification; no comparisons to NAATs available
Hologic ATV	<8 h	Tigris or Panther automated system	Clinician-obtained vaginal swabs, endocervical swabs, or endocervical samples in PreservCyt medium; female urine on Tigris system only	Chlamydia/gonorrhea	$$$	++++	CLIA high complexity, RNA amplification
BD TVQ	<8 h	BD Viper XTR automated instrument	Female urine, patient-obtained vaginal swabs, endocervical swabs	Chlamydia/gonorrhea	$$$	++++	CLIA high complexity, DNA amplification
Cepheid	60–90 min	GeneXpert instrument (variable module numbers available)	Patient-obtained vaginal swabs, endocervical swabs, female and male urine specimens	Chlamydia/gonorrhea	$$$$	++++	CLIA moderate complexity, DNA amplification; pending FDA approval for use in United States

[a] Costs vary by location and laboratory testing volume. Relative costs are shown as higher or lower (more or fewer dollar signs) than other tests, assuming that all factors are equal.

[b] Published sensitivity estimates may be misleading since those estimates are dependent on the sensitivity of the comparator assays. Assays compared only to microscopy or culture may have a high sensitivity estimate, but the estimate compared to a NAAT may be substantially lower. Thus, relative performance is shown here with more plus signs indicating higher sensitivity.

ella vaginalis, and *T. vaginalis* DNA in a single vaginal swab. The test is designed for use only with samples from women with a vaginal discharge. The test is rapid (results are available in <1 h) and is run on a small, assay-specific instrument. The assay can be performed in clinical or site laboratories accredited to perform CLIA moderate-complexity testing. The performance of the assay for detection of *T. vaginalis* in women with vaginitis is better than that of microscopy (28) but significantly poorer than that of NAATs. The real niche for this assay is disentangling the causative agents of vaginal discharge in populations where STIs (e.g., chlamydia or gonorrhea) are not prevalent. The inability to utilize this test for screening among high-risk, asymptomatic women and the lack of companion chlamydia/gonorrhea tests on the same platform limit this test's applicability in many settings.

NAATs have become the recognized gold standard for the screening and diagnosis of *C. trachomatis* and *N. gonorrhoeae* (29). Annual chlamydia screening is recommended for all women <25 years old, regardless of behavioral risk factors or symptoms, in the United States and in many European countries. Recommendations for screening for gonococcal infections are tied to specific behavioral risk factors rather than generalized screening because of the much lower prevalence rates of this pathogen. As mentioned above, targeted screening of women in high-risk populations for trichomonas infection is recommended (7). Despite these differences in recommendations, particularly in the United States, common practice is to bundle chlamydia and gonorrhea testing for women who are eligible for chlamydia screening. Over the last 5 years, several manufacturers of chlamydia/gonorrhea NAATs have produced trichomonas NAATs that can be performed with the same sample (a vaginal swab is the sample of choice, but endocervical samples and female urine are cleared sample types for some assays). As a result, STI control programs now have the opportunity to provide screening and diagnostic services and simultaneously generate improved epidemiologic data regarding infection with *T. vaginalis* in various populations. Below are descriptions of several currently available NAATs that utilize various amplification technologies. Because of the rapidly changing landscape of infectious disease diagnostics, this list is not exhaustive but focuses on assays for which data are available at this time. Further, specific performance characteristics are not provided here. The rationale for this is that sensitivity and specificity estimates are only as good as the tests to which a new assay is compared. Thus, microscopy may have appeared to be highly sensitive compared to nothing but clinical observation. However, we now know that microscopy performs poorly compared to newer tests. The inherent biases in assay evaluation are numerous and make across-study comparisons difficult to the point that they are nearly uninterpretable. While exact-point estimates have little meaning, it is clear that NAATs outperform all other classes of tests for *T. vaginalis*.

The first NAAT for detection of *T. vaginalis* to be approved by the Food and Drug Administration (FDA) in the United States is the Aptima TV assay (Hologic, San Diego, CA). The assay is an adaptation of the Aptima Combo 2 Chlamydia/Gonorrhea test, thus allowing the utilization of samples collected for chlamydia/gonorrhea screening (30). Clinician-collected vaginal swabs, endocervical swabs, and endocervical specimens in PreservCyt (Hologic, San Diego, CA), a liquid-based cytology medium, are approved sample types for the assay. The technology is based on rRNA extraction from specimens, followed by transcription-mediated amplification of captured rRNA. The use of this target provides a natural boost to the limit of detection since 10^2 to 10^3 copies exist within each organism. Amplified products resulting from isothermal amplification are detected by a chemiluminescent reaction measured with a luminometer. The assay may be performed with either the Tigris DTS system or the Panther system, both of which are fully automated and can produce 275 to 400 results in one 8-h work shift. Testing is separate from chlamydia/gonorrhea testing, such that the original specimen must be sampled twice to obtain results for all three organisms, but testing can be done simultaneously on the Panther system so that all results are available at the same time. The performance of this assay is excellent, with sensitivity and specificity estimates of >90% and >95%, respectively (30).

Another high-throughput, fully automated system with an approved assay for *T. vaginalis* is the Viper XTR system, which performs the BD ProbeTec *Trichomonas vaginalis* Q^x (TVQ) assay (BD, Sparks, MD). This assay is based on strand displacement amplification (SDA) of DNA extracted from patient samples with ferric oxide (31). SDA is an isothermal process of real-time amplification and detection of light emission with excellent sensitivity and specificity (>95% and >99%, respectively) (31). The Viper platform can generate >700 results in a single work shift and can test for chlamydia/gonorrhea and trichomonas infections in a single draw from the patient sample container (i.e., a single DNA elution step can provide all three results). In addition to patient-obtained vaginal swabs and endocervical samples, this assay has an FDA claim for female urine. While female urine is not the ideal sample type for trichomonas (or chlamydia/gonorrhea) infection testing (29), it is a commonly obtained sample type, making this a useful advantage.

While high-throughput platforms in reference laboratories may help realize cost savings, there may be associated shipping and time costs that would make testing in smaller, local labs an attractive option. The Affirm assay mentioned above is an example of a test (CLIA moderate) that might be used in an onsite laboratory. However, as mentioned, this assay is for use only with samples from women with vaginitis. NAATs are rapidly becoming available that are intended to fill this testing niche and provide the enhanced sensitivity associated with NAATs. These assays are important to STI control efforts and will shift the current paradigm of who is tested and in what settings. For women with signs or symptoms of infection, these assays may be useful because of their high sensitivity and relatively rapid time to results. For example, a woman with pelvic pain but no vaginal discharge would not be eligible for testing with the Affirm assay but could benefit from NAAT use in an emergency department (ED) or correctional facility setting, where a 60- to 90-min wait for results would not be unreasonable.

One such assay that received FDA approval in 2015 is the AmpliVue Trichomonas assay (Quidel, San Diego, CA). This assay utilizes isothermal helicase-dependent amplification (32) to detect trichomonas DNA with a lateral-flow readout. A small, assay-specific analyzer is required, and this assay is designated a CLIA moderate-level test. Results are available within 1 h, and the sensitivity and specificity appear to be similar to those of culture. It should be noted that no publications are available regarding comparisons between this test and other NAATs, so the sensitivity data in the package insert, which were obtained in comparison with those of the less sensitive microscopy and culture methods, should be taken as a potential overestimate. Unfortunately, this assay cannot be run in conjunction with chlamydia/gonorrhea testing on the same platform, which somewhat limits its applicability in many settings.

At this time, the Xpert TV assay (Cepheid, Sunnyvale, CA) has not

yet been approved by the FDA, but it is available with a CE mark in Europe and is expected to be available in the United States in the near future. This assay uses a real-time PCR system in a disposable cartridge. Instruments range from a single-cartridge module to 80-cartridge instruments. The throughput varies on the basis of the size of the instrumentation, with each cartridge requiring approximately 60 min to generate trichomonas results. The Xpert system has an advantage in that the menu for this platform is broad and flexible since all of the assays are performed within cartridges. Thus, a trichomonas cartridge can be running while a chlamydia/gonorrhea cartridge is running in the next module. The menu extends beyond STIs to a broad range of pathogens, making this instrument ideal for low-throughput settings that choose to consolidate a large number of tests on a single platform. For example, an ED may use this system to generate STI results for a patient presenting with complaints related to sexual health while simultaneously running methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* testing for other patients. Further, this assay has a claim in Europe and will, it is hoped, have one in the United States for male urine, making this the only platform on which testing can be provided for men. This is relevant since the rate of infection among men who are sexual contacts of women with trichomonas infections has been reported to be as high as 70% (16).

T. vaginalis infection has been a largely ignored STI for many years. However, over the last decade, epidemiologic research has consistently demonstrated associations with avoidable negative health outcomes. While the rationale for not adding *T. vaginalis* to the list of notifiable diseases in the United States is sound (9), there remains a need for improved testing opportunities, particularly for women. In many settings, although there is no recommendation from the CDC, when screening for chlamydia/gonorrhea is performed during the first trimester of prenatal health care, trichomonas infection screening is now being added. This screening has been added in large part as a result of the convenience of being able to order multiple tests of a single patient sample. The data to support this screening are becoming stronger as more high-quality tests are being used and more accurate data are being generated (20). Further, in most obstetrics and gynecology and family planning settings in the United States, the rate of trichomonas infection is substantially higher than that of gonorrhea and once providers have access to data about their specific patient populations, they are very interested in having access to this testing.

Many POC and low-throughout options that enable local testing are in development. Those that will have the greatest impact are tests that have the option of being run with chlamydia/gonorrhea testing, have high sensitivity, and produce results rapidly. As with all medical testing, cost will play a key role in uptake, but expanding competition within the market will help to keep costs reasonable. Clinic flow designs that can capitalize on patient-obtained samples and routine test ordering may be able to take advantage of a test-and-treat paradigm within a routine sexual health care or urgent-care visit. Availability of testing for men who are sexual partners of women with trichomonas infections, which is important given the high rate of infection concurrency within sexual partnerships (16), or men who have urethritis will likely increase in demand if that testing can be provided as a near-patient rapid test. Screening in regions such as the southeastern United States, where both trichomonas prevalence and HIV incidence rates among black women are the highest in the country, may provide an additional tool in our HIV prevention toolkit (23). Thus, trichomonas testing is here to stay and will only become more frequently utilized in the future as the assays available for that testing continue to improve in accuracy and convenience to both patients and providers.

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Barbara Van Der Pol has been involved in laboratory-based epidemiologic and behavioral research in the field of STIs and HIV for more than 30 years. She has extensive experience with the development and evaluation of molecular diagnostic assays and is internationally recognized as an expert in this field. She participated in writing the CDC STD Diagnostic Laboratory Guidelines and the WHO STD/HIV Laboratory Diagnostics Manual. She has also evaluated novel methods for STI detection and developed molecular diagnostic assays for the identification of organisms for which no FDA-approved assays exist. Her laboratory developed and evaluated a molecular assay for the detection of *T. vaginalis* DNA 12 years before such an assay became commercially available. She also assessed the feasibility of using self-obtained vaginal swabs collected in nonclinical settings for the detection of *C. trachomatis* and *N. gonorrhoeae* more than 15 years ago. This is now the CDC-recommended optimal sample type for the diagnosis of these infections. More recently, she demonstrated the feasibility of using patient-collected anorectal samples, again in a nonclinical setting, for chlamydia and gonorrhea testing. In addition to her work in the United States, she has spent 2 decades working in sub-Saharan Africa and the Caribbean region providing technology transfer, capacity building, and quality improvement training.