Advances in Laboratory Detection of *Trichomonas Vaginalis* (Updated)

Trichomonas vaginalis is the most prevalent non-viral sexually transmitted infection in the United States and worldwide based on estimates by the WHO and published studies.[1] Trichomoniasis is a common curable sexually transmitted disease caused by this protozoan pathogen. However, it is not reportable nor a nationally notifiable condition. Available diagnostics for *T. vaginalis* range from basic microscopy to nucleic acid amplification assays. Considerations for public health laboratories in choosing an appropriate diagnostic should include the sensitivities and specificities of each assay, as well as costs.

Clinical Information

Trichomoniasis can cause urethritis in men and vaginitis in women, although the majority of infections are asymptomatic.[2] Infection is associated with increased acquisition and transmission of HIV and other STDs[3-5] and linked with pre-term delivery of a low birth weight infant.[6] To reduce symptoms and signs and potentially reduce transmission of trichomoniasis the recommended treatment is a nitroimidazole antibiotic (e.g., metronidazole or tinidazole,) usually in a single dose.[7] Additionally, all sexual partners of infected individuals should be treated at the same time, to prevent reinfection.[7] However, even with this recommendation, because the reinfection rate for women is high, retesting of women should be performed within three months of initial treatment.[7-9] Low level *in vitro* resistance to nitroimidazoles has been reported infrequently and correlation with clinical outcomes is inconsistent.[10]

Epidemiology

An estimated 3.7 million women and men are infected with *T. vaginalis* in the United States.[11] Among women aged 14–49 years participating in the National Health and Examination Survey (NHANES) in 2001-2004, the overall prevalence of *T. vaginalis* infection measured by PCR was 3.1%, and varied considerably by race: 1.3% for non-Hispanic white women, 1.8% for Mexican American women, and 13.3% for non-Hispanic black women. Other significant correlates of infection included increasing age, a greater number of lifetime sex partners, lower educational level, poverty and douching.[4]

Diagnostic methods

There has been a movement in the STD testing realm to have patients collect their own specimens, called self-collection (SC) in addition to or in some cases in lieu of the clinician collection (CC). Therefore, for some tests self-collected specimens are delineated as a specific FDA cleared specimen type, differentiated from a clinician collected specimen. If there was a delineation we have indicated so by using the abbreviations above. Additionally many of these assays have been FDA cleared for use with specific collection devices and/or transport media that must be used in accordance with the package insert.

The traditional diagnostic method for trichomoniasis has been **wet mount** with microscopic visualization of motile *T. vaginalis* parasites on slide preparations from vaginal or urethral secretions. Ideally, specimens should be examined within minutes (<10) to see motile parasites, which are diagnostic. Wet mount is an inexpensive specific diagnostic test; however, sensitivity ranges from 36-70% at best, and varies by evaluator and how promptly the slide is interpreted.[12-18]

Culture has been considered the gold standard for diagnosis of trichomoniasis with a specificity approaching 100%, but it is not widely used and its sensitivity can be as low as 70%–85%.[13] Clinical specimens can be inoculated into transport systems such as Amies gel medium to maintain viability for up to 24 hours at room

temperature. Culture systems such as InPouch TV (Biomed Diagnostics, Inc., White City, OR) allow for direct inoculation, transport, culture and microscopic examination. Such systems are useful when immediate transportation of specimens to the laboratory is not available. The specimen should be inoculated as soon as possible (within an hour of collection) to maintain viability of the organism.

Neither conventional nor liquid-based **Papanicolau (Pap) smears** are suitable for routine screening or diagnosis of *T. vaginalis*, as both false positives and false negatives occur. However, the presence of these parasites may be an incidental finding.[7]

The OSOM (formerly Xenostrip) Trichomonas Rapid Test (Sekisui Diagnostics, Framingham, MA) is an immunochromatographic capillary-flow enzyme immunoassay dipstick test and the only **rapid antigen test** commercially available in the U.S. It is performed on vaginal secretions with results available within 10 minutes. This test is FDA cleared for females and CLIA waived and is amenable to use in a point-of care setting.

The Affirm VP III (Becton Dickinson, San Jose, CA) is an FDA-cleared **nucleic acid probe** test for the diagnosis of *T. vaginalis* as well as *Gardnerella vaginalis* and *Candida albicans* in females. This test can be run and produce results in 45 minutes and could be performed in a point-of-care setting but is not CLIA-waived and must be performed in a lab capable of performing CLIA moderate complex tests. This assay detects and identifies DNA using specific capture probes in a nucleic acid hybridization that is detected based on an enzymatic color change.

Several highly sensitive **nucleic acid amplification tests** (NAATs) are available for detection of *T. vaginalis* in symptomatic and asymptomatic women (unless otherwise noted). Many of these assays require the use of specific specimen collection kits to meet the FDA labeling and intended use so users should refer to the package insert for those details.

The AmpliVue Trichomonas assay (Quidel Corp., San Diego, CA) is FDA cleared for use with vaginal swabs (CC) for the qualitative detection of *T. vaginalis* nucleic acid. This assay uses an isothermal amplification called helicase dependent amplification with nucleic acid probes and a disposable lateral flow detection device. It can be performed in 50 minutes with minimal basic equipment in a CLIA moderately complex setting.

The APTIMA TV assay (Hologic, San Diego, CA) is FDA cleared for two different platforms. The assay for the Panther System may be used with vaginal (CC) and endocervical (CC) swabs and endocervical specimens collected in PreservCyt Solution. The assay for the Tigris DTS System includes all of the above as well as female urine. Both of these assays utilize a target capture, transcription mediated (TMA) and hybridization protection assay for detection of TV ribosomal RNA.

The BD MAX CT/GC/TV Assay (Becton Dickinson, Franklin Lakes, NJ) received FDA clearance in September 2016. The assay incorporates automated extraction followed by real-time PCR for the direct, qualitative detection of *C. trachomatis*, *N. gonorrheae* and *T. vaginalis*. For detection of *T. vaginalis* the assay may be used with vaginal (SC) or endocervical (CC) swab specimens and urine (F). In October 2016, the BD Max Vaginal Panel (Becton Dickinson, Franklin Lakes, NJ) also received FDA Authorization. Similar to the CT/GC/TV Assay it also incorporates automated extraction followed by real-time PCR to detect microorganisms responsible for bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis (TV).

The Solana® Trichomonas assay (Quidel Corp., San Diego, CA) is FDA cleared for use with vaginal swabs (CC) and female urine for the qualitative detection of *T. vaginalis* nucleic acid. This assay uses an isothermal amplification called helicase dependent amplification with fluorescence detection. It can be performed in 30 minutes on 1 to 12 specimens using their propriety Solana small benchtop instrument in a CLIA moderately complex setting.

The *Trichomonas vaginalis* (TV) Q^{x} Amplified DNA Assay (Becton Dickinson, Franklin Lakes, NJ) is FDA cleared for use with vaginal (SC), or endocervical (CC) swab specimens and female urine. The assay uses a strand displacement amplification for the direct detection of TV DNA.

Cepheid (Sunnyvale, CA) obtained FDA clearance for a qualitative *T. vaginalis* assay in 2015 with their Xpert TV assay. Approved specimen types for this assay include vaginal (SC) and endocervical (CC) swab specimens and both female and male urine. This test uses real-time PCR to detect TV genomic DNA.

NAATs testing for *T. vaginalis* in females at high risk for STD results in significantly higher detection rates over that of wet prep microscopy. In a recent study conducted in an STD clinic in Alabama, the prevalence of *T. vaginalis* in women was 19.6% by wet mount versus 27% by NAATs. Thus, significantly more infections were detected using NAATs resulting in enhanced treatment and partner notification. In this same study, NAATs testing in males revealed a prevalence of 9.8% compared to no male cases detected prior to the development of this technology again resulting in enhanced disease control efforts.[19]

The superb sensitivity and specificity of NAATs have now made them the gold standard for diagnosis of trichomoniasis. The only FDA-cleared NAAT for use with male specimens is the Xpert TV Assay (Cepheid). However, other methods could be used with male specimens after an internal validation process has been completed by the laboratory.

Screening and Treatment

Current recommendations for T. vaginalis testing and screening, along with detailed clinical treatment recommendations, can be found in CDC's STD Treatment Guidelines, available online at http://www.cdc.gov/std/treatment.

Table 1: Overview and characteristics of diagnostic assays for *Trichomonas vaginalis**

(underlined tests are hyperlinked to online resources)

Diagnostic Test	Technique	Time to Result	Specimen Type	Sensitivity (with 95% CI)	Specificity (with 95% CI)	Comments
Wet mount	Microscopic visualization	Minutes	Vaginal or urethral secretions	36-70%[a, 12-18]	99.8-100%[a, 12-18]	Traditional; inexpensive; operator-dependent; must be performed within minutes
Culture (Diamond's or In-Pouch™)	Culture	1-5 days	Vaginal or urethral swab; urine (F), semen	75-95%[a, 12-16]	100%[a, 12-16]	Diagnostic gold standard before NAATs
OSOM Trichomonas Rapid Test	EIA	10 minutes	Vaginal swabs, saline solution for wet mount prep of vaginal swab	75-96% (67-100)[b]	95-99% (92-100%)[b]	FDA cleared and CLIA waived
Affirm VP III	Nucleic acid probe test	45 minutes	Vaginal swabs	89.2-92.8%[b]	98.1-99.9%[b]	FDA cleared; can be used at the point of care; CLIA moderately complex
AmpliVue Trichomonas	NAAT	50 minutes	Vaginal swab (CC)	100% (96.9-100%)[b]	98.2% (97.0-98.9%)[b]	FDA cleared, CLIA moderately complex
APTIMA TV (Panther)	NAAT	Hours	Vaginal (CC) and endocervical (CC) swab, endocervical (CC) specimens in Preserv-Cyt Solution	95.2-100% (88.4-100%) [b]	98.9-99.6% (97.8-99.9%) [b]	FDA cleared, CLIA moderately complex
APTIMA TV (Tigris)	NAAT	Hours	Vaginal (CC) and endocervical (CC) swab, endocervical (CC) specimens in Preserv-Cyt Solution, urine (F)	95.2-100% (88.4-100%)[b]	98.9-99.6% (97.8-99.9%)[b]	FDA cleared, CLIA moderately complex
BD MAX CT/GC/TV Assay	NAAT	Hours	Vaginal (SC) and endocervical swab (CC), urine (F)	92.9-96.1% (87.7-98.2%)[b]	98.9-99.3% (98.0-99.7%)[b]	FDA cleared, CLIA moderately complex
Solana Trichomonas	NAAT	30 minutes	Vaginal swab (CC), urine (F)	95.5-98.3% (90.0-99.5%)[b]	98.2-98.7% (97.1-99.3%)[b]	FDA cleared, CLIA moderately complex
TV Q Amplified DNA	NAAT	Hours	Vaginal (SC)c and endocervical swab (CC), urine (F)	95.5-98.3% (90.0-99.5%)[b]	98.7-99.4% (97.5-99.8%)[b]	FDA cleared, CLIA moderately complex
Xpert TV	NAAT	35 minutes	Vaginal (SC) and endocervical (CC) swab, urine (M,F)	96.4-98.9% (92.7-99.9%)[b]	98.9-99.7% (98.3-99.9%)[b]	FDA cleared, CLIA moderately complex

Abbreviations: EIA: Enzyme Immunoassay, NAAT: nucleic acid amplification test.

[a]Sensitivity and specificity are ranges reported from primary literature articles cited.

[b]Sensitivity and specificity are the ranges reported for the overall performance for the approved specimens within the product insert. Please review the product insert to determine what was being compared (specimen type, reference test, symptomatic vs asymptomatic patients) to calculate these values. For products that test for more than one pathogen, the data is only for the detection of *T. vaginalis*.

[c]Self collected vaginal swabs must be collected in a clinical setting.

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