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Modern diagnosis of *Trichomonas vaginalis* infection

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Abstract

Recent advances in tests for the sexually transmitted protozoan parasite *Trichomonas vaginalis* have increased opportunities for diagnosis and treatment of this important sexually transmitted infection. This review summarises currently available tests, highlighting their performance characteristics, advantages and limitations. The recent development of molecular tests for the detection of *T vaginalis*, including rapid antigen detection and nucleic acid amplification tests, has significantly improved the quality of diagnostics for trichomoniasis, particularly in women. In light of the expanded menu of testing options now available, improved recognition and better control of trichomoniasis are in sight, which should enable the eventual reduction of adverse reproductive consequences associated with *T vaginalis* infection.

INTRODUCTION

In 2008, 276.4 million incident cases of *Trichomonas vaginalis* infections were estimated by WHO, which represent an increase of 11.2% compared with global estimates for 2005.[1] The incidence of trichomoniasis exceeds rates for gonorrhoea and chlamydial infection combined; however, the development of sensitive and specific detection methods for this sexually transmitted protozoan has lagged behind diagnostic testing for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

T vaginalis causes vaginitis and cervicitis in women and urethritis in men; however, infections are often asymptomatic. Trichomoniasis is associated with adverse reproductive sequelae including preterm birth and pelvic inflammatory disease in women and infertility in women and men.[2,3] Furthermore, *T vaginalis* infection increases sexual transmission of HIV, and high rates of trichomoniasis have been reported among HIV-infected women.[4–6] Likely resulting from disrupted innate immune responses and increased inflammation in infected genital mucosae, trichomoniasis doubles or triples the risk of acquiring HIV infection.[2] Modelling studies indicate that *T vaginalis* may account for nearly 20% of HIV transmission events from HIV-infected individuals when the prevalence of trichomoniasis is high.[7]

Trichomoniasis is not a reportable sexually transmitted infection (STI) in many countries, including the USA, and comprehensive guidelines for testing are lacking or slowly

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emerging. In addition, wet mount microscopy, which is the diagnostic method traditionally used to identify infection mainly in women, is insensitive. Thus, *T vaginalis* infection is underappreciated by healthcare providers and patients alike. However, recent advances in diagnostic tests to detect trichomonas offer hope for improved recognition and treatment, which may lead to better control of this important STI. Herein we review the advantages and limitations of the most commonly available contemporary methods for the diagnosis of *T vaginalis* infection.

CLINICAL PRESENTATIONS OF TRICHOMONIASIS

The signs and symptoms of trichomoniasis are similar to those caused by other STIs and bacterial vaginosis. Clinical presentations of trichomoniasis in women range from asymptomatic to severe vaginitis characterised by diffuse, malodorous discharge or urethritis characterised by dysuria. Cervicitis characterised by petechial haemorrhages on the ectocervix (the so-called `strawberry cervix') may distinguish trichomoniasis from other causes of cervicitis, but this is not frequently identified. The clinical presentation of trichomoniasis in men exhibits a similar spectrum ranging from asymptomatic infection to urethritis characterised by urethral discharge and dysuria. Asymptomatic *T vaginalis* infections are common. Studies in sexually transmitted disease clinic populations suggest that approximately 25% of women[89] and 40%–75% of infected men with trichomoniasis are asymtpomatic.[810] Because the clinical manifestations of trichomoniasis are non-specific, and infection is very often asymptomatic, accurate diagnosis of *T vaginalis* infection requires appropriate laboratory testing.

LABORATORY TESTING FROM MICROSCOPY TO NUCLEIC ACID AMPLIFICATION

Microscopy

Microscopic examination of a wet mount preparation of vaginal secretions mixed with normal saline is the most common diagnostic evaluation for *T vaginalis* infection in women. Direct observation of the pear-shaped trichomonads with their characteristic jerky or tumbling motility is considered 100% specific for *T vaginalis*. Studies comparing wet mount microscopy with highly sensitive molecular detection tests document the poor sensitivity of microscopy, which ranges from 44% to 68%, even with experienced microscopists and prompt examination of vaginal specimens.[11–13w1w2] Delays as short as 10–30 min between specimen collection and microscopic examination can dramatically reduce the sensitivity of the test.[14] In addition, suboptimal specimen storage or transportation conditions, especially temperatures below 22°C, further reduce parasite motility and thus wet mount sensitivity. Key features of microscopy and other available tests are summarised in table 1.

The microscopic detection of trichomonads is often reported as an incidental finding in Papanicolaou (Pap) smears of cervical specimens. The conventional Pap smear technique is considered unreliable for diagnosis of *T vaginalis* infection with poor sensitivity and specificity, and confirmatory testing with a more sensitive and specific test is recommended for asymptomatic women with trichomoniasis diagnosed by conventional Pap. However, recently developed liquid-based Pap tests appear to be more accurate for microscopic identification of *T vaginalis*. Reported sensitivities range from 60% to 96% and specificities from 98% to 100%,[1516] suggesting that treatment of trichomoniasis identified in liquid-based Pap test pathology reports, without additional testing, may be justified.

Microscopic examination of vaginal wet mounts and liquid-based Pap specimens are widely available and relatively inexpensive. Wet mount microscopy has the advantage of providing immediate results as a point-of-care test; liquid-based Pap test results are typically available

several days after specimen collection. Unfortunately, microscopic examination of male urethral specimens or urine sediment is unreliable for detection of *T vaginalis* infection in men due to the lower organism burden.[22]

Culture

Biological amplification of *T vaginalis* in liquid culture provides improved sensitivity over direct microscopic observation. Culture techniques include the use of Diamond's modified medium, available in glass tubes from commercial microbiological media suppliers, and the InPouch TV test (Biomed Diagnostics, Oregon, USA). Specimens from women (vaginal swabs) or men (urethral swabs or urine sediment) should be used to immediately inoculate the culture medium less than 1 h after collection. Cultures are incubated at 37°C and examined microscopically each day for up to 5 days until motile trichomonads are observed. Cultures from women with trichomoniasis are usually positive within the first 3 days after inoculation. However, cultures from men should be examined daily for 5 days or longer before being considered negative, as extended incubation times are often required to permit growth of detectable numbers of organisms from specimens from male specimens.[22]

InPouch TV is a self-contained culture pouch made of oxygen-resistant, optically clear plastic that can be microscopically examined directly, eliminating the need to remove material from the culture for daily examination. InPouch TV can be stored at room temperature before use, and inoculated pouches can remain at room temperature up to 48 h before incubation at 37°C (Biomed product insert). Diamond's medium must be stored at 4°C before use, warmed to room temperature before inoculation and cultures should be incubated immediately at 37°C under anaerobic conditions.

Compared with highly sensitive nucleic acid amplification tests (NAATs) the sensitivity of culture ranges from 44% to 75% for detection of *T vaginalis* in specimens from women.[1112w1w3] In men, the sensitivity of culture ranges from 40% to 56% for detection of *T vaginalis*;[22w4] urine from men is more sensitive than a urethral swab specimen for culture.[22] Based on visualisation of viable, motile trichomonads, culture is 100% specific for detection of *T vaginalis*. Liquid culture media are relatively inexpensive, but the cost of culture is increased by the requirement for daily examination by a trained microscopist, and final results may take up to a week.

Rapid diagnostic tests

Traditional wet mount microscopy and culture require rapid specimen handling, processing and transport conditions to preserve viable, motile organisms. In contrast, several recently developed non-culture tests that detect *T vaginalis* antigens or nucleic acids allow for extended time between specimen collection and testing and more flexible sample storage temperatures. Commercially available antigen detection tests include the OSOM Trichomonas Rapid Test (Sekisui Diagnostics, California, USA) and the Tv latex agglutination test (Kalon Biological, Surrey, UK). OSOM is a US Food and Drug Administration (FDA)-cleared point-of-care, immunochromatographic strip test that uses specific antibodies to detect trichomonas protein antigens. When present, *T vaginalis* antigens bind the antibodies resulting in the formation of a blue line on the test strip. Sample processing and testing require no instrumentation, and results are available in 30 min or less. The Kalon Tv latex agglutination test is not registered with the US FDA and does not bear a Conformité Européenne (CE) mark for use as a diagnostic device in the European Union (EU); it is a point-of-care test that uses latex beads coated with specific antibody to detect trichomonas protein antigens. When present, *T vaginalis* antigens cause the beads to agglutinate on a glass slide. Sample processing and testing require no instrumentation, and results are available in 10 min or less.

Affirm VPIII (Becton Dickinson, Maryland, USA) is a non-amplified nucleic acid probe hybridisation test for detection of *T vaginalis, Gardnerella vaginalis* and *Candida albicans*. Affirm VPIII is a US FDA-cleared test and bears the EU CE mark. Affirm uses specific oligonucleotide probes to detect *T vaginalis* (also *G vaginalis* and *C albicans*) nucleic acids; sample processing and testing require a heating block and a processor instrument. The test can be done in approximately 1 h, but in practice, samples are usually batched, and the moderate complexity Affirm test is not routinely used as a rapid test.[23]

The sensitivities of the non-culture, non-amplified tests described above are similar to culture and consistently higher than wet mount microscopy. and range from 40% to 95% depending on the specific test and the reference standard.[91317–21] These tests are highly specific for *T vaginalis* with clinical specificities ranging from 92% to 100%.[91317–21] OSOM, Tv latex and Affirm are all intended for use with vaginal swabs from symptomatic women or women with suspected exposure to trichomoniasis; none of these tests has been evaluated for screening asymptomatic women or for diagnosis of trichomoniasis in men.

Nucleic acid amplification tests

As with other STIs, the advent of highly sensitive and specific NAATs has provided critical new tools for diagnosis of infections with *T vaginalis*. NAATs include PCR, transcription-mediated amplification (TMA) and other technical variations on a biochemical theme characterised by the replication and amplification of millions of copies from specific individual DNA or RNA target sequences. Thus, the analytical sensitivity of NAATs is inherently greater than that of microscopy, culture, antigen detection or nucleic acid probe assays, which detect existing organisms or their constituents. The high analytical specificity of NAATs stems from the use of nucleotide primer and probe sequences that are unique to the target organism. Standard procedures during the development of inhouse and commercial NAATs for diagnosis of STIs include verification that the tests do not detect pathogens and common microbial inhabitants of the urogenital tract other than the intended target and that the presence of other organisms does not interfere with detection of the target.

Several inhouse PCR assays have been described and validated in individual laboratories for detection of *T vaginalis* in women and men.[12w1w3–w7] The TMA-based APTIMA *T vaginalis* assay (Hologic Gen-Probe Inc, California, USA) is the first commercial NAAT to receive the EU CE mark and US FDA clearance for in vitro diagnostic use to detect *T vaginalis* in women.[24] APTIMA uses specific rRNA target capture, TMA and detection of amplified products by a hybridisation protection assay and requires a high complexity instrumentation system and trained laboratory personnel. Consequently, APTIMA is not a point-of-care test, and costs are correspondingly higher than for many of the non-NAATs. Specimens are typically obtained and placed in APTIMA transport medium at the site of collection and sent to large laboratories using the automated TIGRIS DTS System, where results for hundreds to thousands of specimens are reported daily. A manual DTS instrumentation system is also available for smaller laboratories, and APTIMA assay clearance for use with an automated PANTHER System for small to mid-sized laboratories is forthcoming.

The increased sensitivity of NAATs compared with the non-amplified tests, combined with convenient specimen processing that preserves nucleic acids but not viable organisms, has several important advantages for diagnosis of trichomoniasis in both men and women. *T vaginalis* NAAT sensitivities range from 76% to 100%,[12132124w1–w7] making these tests suitable for screening and testing asymptomatic female and male patients, in whom trichomoniasis is typically characterised by relatively low organism burdens. A variety of urogenital specimens can be used with NAATs, including non-invasive urine and minimally

invasive self-collected vaginal swabs in addition to clinician-collected vaginal, urethral and endocervical swabs; endocervical specimens collected for liquid-based Pap cytology can also be used for NAATs. *T vaginalis* is considered to be primarily a vaginal pathogen, in contrast to *N gonorrhoeae* and *C trachomatis*, which typically infect the endocervix. However, all three organisms can be readily detected in either an endocervical or vaginal specimens using highly sensitive NAATs.[24]

Because viable organisms are not required, specimen storage, processing and transport allow for a wider range of temperatures and time intervals between specimen collection and testing. Thus, NAATs can facilitate testing in diverse settings from clinical to non-clinical venues and can be applied for screening in population-based epidemiological studies. Specimens obtained for *T vaginalis* NAATs are appropriate and usually contain sufficient quantity for detection of other STIs including *N gonorrhoeae* and *C trachomatis*. Thus, combined testing for trichomoniasis, gonorrhoea and chlamydial infection is feasible from a single urogenital specimen.[w8]

The potential of NAATs to detect non-viable organisms imposes limitations on their use as tests of cure for trichomoniasis. Although NAATs may remain positive for several days, these tests are generally negative 2 weeks after successful treatment for *T vaginalis* infection.[25] The failure to preserve viable organisms in specimens processed for NAATs also limits the potential for antimicrobial susceptibility testing for *T vaginalis* and other pathogens for which the molecular basis of resistance is not known. Thus, although NAATs may eventually supplant less sensitive tests for diagnosis of *T vaginalis* infection, culture will continue to be important in cases of persistent infection or suspected treatment failure for which antimicrobial susceptibility testing of clinical isolates may be warranted.

SPECIMEN COLLECTION AND TESTING STRATEGIES FOR *T VAGINALIS* DIAGNOSIS

The choice of specimen, diagnostic test and testing strategy for detection of *T vaginalis* will naturally depend on the patient population, clinical setting, available laboratory facilities and cost considerations. In clinical settings where there is a high prevalence of STIs and there are resources to support molecular diagnostic laboratory testing, collection of vaginal or endocervical samples from women and urethral swabs or urine from men for NAATs will likely provide optimal diagnosis of *T vaginalis* infections. If universal screening is not feasible among all sexually active men and women presenting for STI evaluation, targeted testing should be considered for women presenting with vaginitis, cervicitis or urethritis; men with urethritis; and sexual partners of persons diagnosed with trichomonal infection, preferably with NAATs.

In settings where physical examinations for STI evaluation are limited (eg, community-based health centres, school-based clinics, correctional facilities, outreach venues or home screening), non-invasive urine or self-collected vaginal swabs may be preferable. Only non-culture-based testing is feasible in such situations because viable organisms cannot survive the inevitable delays in specimen transport to a laboratory facility. Urine specimens could be collected in those settings from both men and women, but testing would require transport to a qualified laboratory for NAATs. Point-of-care testing might be practical for self-collected vaginal swabs in such situations. Currently available rapid tests are not designed for use by the general public and generally require some operator training. However, home specimen collection and testing using a rapid antigen detection test have been shown to be feasible, and women who were provided detailed instructions for vaginal swab collection and testing produced reliable results.[w9]

Numerous websites have been developed to offer internet-based STI testing. Most offer self-collection kits and testing options for gonorrhoea and chlamydial infection, and increasingly for trichomoniasis. Although a recent survey showed that the accuracy of STI tests offered through internet sites is highly variable, mailed-in specimens tested in well-qualified laboratories provide accurate test results.[w10] The public health website http://www.iwantthekit.org offers *T vaginalis* testing from self-collected vaginal swabs from women and urine or self-collected penile swabs from men in select areas of the USA.[2627w11] In men, the sensitivity of NAATs from self-collected penile swabs is higher than urine for *T vaginalis* detection.[28w11]

In settings where molecular testing is not available, diagnosis of *T vaginalis* infection remains problematic because wet mount microscopy alone performs poorly for the diagnosis of trichomoniasis in women. In those settings, testing algorithms in which vaginal specimens from wet mount-negative women are tested using a rapid antigen detection test and/or culture can substantially improve detection of *T vaginalis*.[29w12] Wet mount microscopy and current rapid antigen detection tests are not suitable for diagnosis of trichomoniasis in men; inoculation of culture media with multiple specimens (eg, a combination of urine sediment and a urethral swab) increases the sensitivity of culture for detection of *T vaginalis* infection and should be considered.[30]

SUMMARY AND CONCLUSIONS

Once regarded as merely a nuisance in women, *T vaginalis* infection can cause various clinical presentations in both men and women and is a risk factor for HIV transmission. In response to increasing recognition by clinicians, researchers and policymakers of the importance of this widespread STI, new molecular diagnostic tests with improved sensitivity have been developed and validated by manufacturers and in clinical and research laboratories throughout the world. With enhanced awareness, availability and application of these modern diagnostic tests, better detection and treatment of trichomoniasis in women and in their sexual partners can be achieved with eventual reduction of adverse reproductive consequences associated with *T vaginalis* infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key messages

- A variety of diagnostic tests are now available to detect *Trichomonas vaginalis* infection in women; fewer options are available for use in men.
- Rapid point-of-care tests with improved sensitivity compared with wet mount microscopy should facilitate testing and treatment of women in clinical and non-clinical settings.
- *T vaginalis* nucleic acid amplification tests enable incorporation of testing for this infection in settings where molecular diagnostics for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are already in place.
- Molecular diagnostic tests perform well with self-collected specimens and can be used in innovative screening and testing programmes including internet recruitment.

Table 1

Key features of commonly available diagnostic tests for *Trichomonas vaginalis* infection

Category	Test	Sensitivity range* (%)	Specificity range* (%)	Advantages	Limitations	References
Direct microscopy	Wet mount	44–68	100	Same day results, inexpensive	Low sensitivity, requires trained microscopist, not for use in men	11–13w1w2
	Conventional Pap	44–79	83–99	Convenient for women undergoing cervical cancer screening	Low sensitivity and specificity, requires confirmatory testing, requires trained microscopist, several days for results, not for use in men	1516
	Liquid Pap	60–96	98–100	Improved sensitivity and specificity versus conventional Pap	Requires trained microscopist, several days for results, not for use in men	1516
Culture	Diamond's modified medium or InPouch	44–75	100	Improved sensitivity versus microscopy, antimicrobial susceptibility testing possible	Requires trained microscopist, incubator and controlled temperature transport, up to a week for results	1112w1w3w4
Non-amplified molecular tests	OSOM rapid antigen test	77–98	99–100	Same day results, minimal training required, no equipment needed, specimen transport delays tolerated	Not for use in asymptomatic women or in men	1317–19
	Kalon TV agglutination	55–99	92–100	Same day results, minimal training required, no equipment needed, specimen transport delays tolerated	Not for use in asymptomatic women or in men	920
	Affirm VP III nucleic acid probe hybridisation	64	100	Same day results possible, *Gardnerella* and yeast detection included, specimen	Moderate complexity, some training and equipment required, not for use in	21

Category	Test	Sensitivity range* (%)	Specificity range* (%)	Advantages	Limitations	References
				transport delays tolerated	asymptomatic women or in men	
NAATs	APTIMA TV	88–100	98–100	Highly sensitive and specific, specimens compatible with testing for other STIs, specimen transport delays tolerated, performs well with specimens from men	Expensive, requires laboratory equipment and highly trained personnel, several days for results, persistent positives following treatment possible	121321w2
	Inhouse PCR	76–100	96–100	Highly sensitive and specific, specimens compatible with testing for other STIs, specimen transport delays tolerated, performs well with specimens from men	Expensive, requires laboratory equipment and highly trained personnel, several days for results, persistent positives following treatment possible	12w1w3–w6

NAAT, nucleic acid amplification test; Pap, Papanicolaou; STI, sexually transmitted infection; TV, *Trichomonas vaginalis*.

*Wide ranges for sensitivity and specificity result from the use of different reference standards in published studies.