Methods for Detection of *Trichomonas vaginalis*

*a report by*

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*Trichomonas vaginalis* is a protozoan pathogen of the human urogenital tract. The World Health Organization (WHO) estimates that trichomoniasis accounts for more than half of all curable sexually transmitted infections (STIs) worldwide.1 Although accurate surveillance data are lacking, there were an estimated five million new cases of trichomoniasis each year in the US during the late 1990s, exceeding similar figures for gonorrhea and chlamydial infections combined.2 The prevalence of *T. vaginalis* has been reported to be as high as 26% among female STD clinic patients and 22% among HIV-positive women.3 Recurrent infections are common in women, predominantly due to untreated sexual partners.4 Transmitted primarily by sexual intercourse, *T. vaginalis* causes vaginitis in women and urethritis in men. While trichomoniasis is well recognised as an aetiological cause of vaginitis, the proportion of non-gonococcal urethritis in men due to *T. vaginalis* has been estimated to be between 11 and 18%.5 A substantial proportion of infections are asymptomatic, necessitating reliable testing methods. Trichomoniasis is also implicated in various other genito-urinary syndromes, including cervicitis, epididymitis and prostatitis.5 Associations between maternal trichomoniasis and premature rupture of the membranes and pre-term delivery have been reported,6–8 and there is mounting evidence of an association with cervical cancer.9–14

Infection with *T. vaginalis* can be a marker for high-risk sexual behaviour, and frequently occurs concomitantly with other STIs, including gonorrhea and chlamydia.5 *Trichomoniasis* is associated with incident herpes simplex virus (HSV)-2 infection15 and with genital HSV-2 shedding in infected women.16 As with other STIs, trichomoniasis in the male or female genital tract is associated with increased sexual transmission of HIV.17–21 Co-infection with *T. vaginalis* and HIV may increase the infectiousness of both organisms.17,22 As the prevalence of trichomoniasis is so high, a large proportion of HIV infections could be attributable to *T. vaginalis* infection in populations where both infections are common.8 Diagnosis of trichomoniasis based solely on clinical signs and symptoms is unreliable because the spectrum of infection is broad and other sexually transmitted pathogens can cause similar signs and symptoms.8 Diagnosis is particularly challenging in men, where infections are characterised by fewer organisms than infections in women.23,24 In this report, we review the performance characteristics, advantages and limitations of currently available *T. vaginalis* detection methods.

**Direct Microscopy**

In clinical practice, laboratory diagnosis of trichomoniasis has previously relied on microscopic examination of a wet-mount preparation of vaginal discharge or male urine sediment. Because of the characteristic shape and unique tumbling motility of live *T. vaginalis* in such preparations, wet-mount microscopy is assumed to have perfect specificity. In expert hands, wet-mount microscopy can be 50–70% sensitive in specimens from women, but the technique is much less reliable in specimens from men.5 Diagnosis by wet-mount requires visualisation of viable, motile protozoa; therefore, specimens must be examined immediately. The sensitivity of wet-mount microscopy can be further reduced as a result of delays between specimen collection and examination.25 Despite its limited sensitivity, wet-mount microscopy may be widely used because it is inexpensive and positive results can be obtained quickly, allowing patients to be treated during a single clinic visit. *T. vaginalis* can also be visualised in fixed vaginal, cervical or urine sediment smears stained using various staining methods, including Gram, Giemsa, Papanicolaou, periodic acid-Schiff, acidine orange, fluorescein and immunoperoxidase. Papanicolaou-stained smears can be used to detect *T. vaginalis* in asymptomatic women during routine examinations.26–28 Detection using the Papanicolaou test has reported sensitivity of 60% and specificity of 95–97%;29,30 however, confirmation of trichomonads using another method is recommended.

**Culture**

Culture, using a variety of liquid and semi-solid media, remains the reference standard for diagnosis of trichomoniasis in women.5,31–34 Culture pouches containing modified Diamond’s medium are commercially available and convenient (InPouch TV, Biomed Diagnostics, White City, OR). After inoculation with a vaginal swab specimen or urine sediment, cultures are incubated for three to five days at 37°C in a 5% CO2 atmosphere and examined daily using microscopy for motile trichomonads. Cultures from women with trichomoniasis are usually positive within the first three days. In studies comparing the detection of *T. vaginalis* using culture and highly sensitive nucleic acid amplification tests, sensitivity estimates for culture...
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range from 69 to 75% with specimens from women. However, because positive cultures may result from the growth of even a few trichomonads in a clinical specimen, this method is more sensitive than wet-mount microscopy and substantially improves detection of T. vaginalis. Vaginal swab specimens transported in Amies gel transport tubes maintain T. vaginalis viability for up to 24 hours prior to inoculation into culture media. Trichomonads from vaginal swab specimens have been shown to remain viable in a small amount of saline for up to 20 minutes prior to culture inoculation. A combined approach of microscopy followed by culture if the wet-mount is negative can increase diagnostic sensitivity in women over microscopy alone. T. vaginalis culture is less sensitive for detection of trichomoniasis in men. Furthermore, cultures inoculated with specimens from men should be incubated and examined daily for the full five days, since they often do not become positive until after three or more days of incubation. Although culture of T. vaginalis in men is preferred over wet-mount microscopy for diagnosis, the optimal specimen for culture is not clear. Studies indicate that sampling multiple urogenital sites substantially increases the detection of T. vaginalis in men. Semen cultures have been shown to be valuable for documentation of infection; infections may be diagnosed by positive semen cultures in the face of concomitant negative cultures from urine, urethral swabs or external genitalia. However, collection of semen for diagnostic purposes is not feasible in most clinics, and microscopic examination of more than one specimen per subject is time-consuming. A practical approach is to combine a urethral swab specimen with a first-voided urine sediment in a single culture.

Rapid Diagnostic Tests
Point-of-care tests for diagnosis of trichomoniasis in women are now commercially available, and they provide rapid and sensitive detection methods during the same clinic visit. Unfortunately, no rapid diagnostic tests are yet available for diagnosis of trichomoniasis in men. The Affirm™ VPII Microbial Identification Test (Becton Dickinson, Franklin Lakes, NJ) is an office-based oligonucleotide probe test that has a sensitivity of 80–90% and a specificity of 95% compared with wet-mount plus culture using vaginal swabs. The OSOM® Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, MA) is an immunochromatographic strip test that detects T. vaginalis antigens in vaginal swabs. Performance characteristics of the OSOM test (sensitivity 83–90% and specificity 99–100%) are superior to wet-mount microscopy and culture favourably with culture and nucleic acid amplification testing (NAAT) (described below). Rapid tests are likely to be particularly important for use in settings where culture and microscopy are not possible, and in populations such as adolescents and patients who are seen in emergency departments, both of whom present challenges to follow-up.

Nucleic Acid Amplification Tests
The development of highly sensitive NAATs for detection of T. vaginalis has lagged behind diagnostic advances for detection of Neisseria gonorrhoeae and Chlamydia trachomatis. Until recently, in-house polymerase chain reaction (PCR) assays were the only available NAATs, and access has been limited primarily to research laboratories. The performance of various PCR primers assessed in clinical studies differs, with reported sensitivities ranging from 80 to 100%. Special challenges arise in evaluating the specificity of NAATs, since the reference standard (usually culture) is often inherently less sensitive than the newer amplification tests. As a result, some positive NAAT results classified as false-positives in comparative analyses are likely to be true positives. Reported specificities of in-house PCR assays for T. vaginalis detection range from 88 to 100%. One NAAT that uses transcription-mediated amplification (APTIMA TV analyte-specific reagents; Gen-Probe, Inc., San Diego, CA) is now commercially available but has not yet been evaluated for clearance by the US Food and Drug Administration (FDA). Validation studies comparing the APTIMA TV assay with in-house PCRs and traditional methods for detection of T. vaginalis from women report sensitivity ranges from 96 to 98% and specificity of 98%. Vaginal swabs are generally more sensitive specimens than urine for NAAT detection of T. vaginalis infections in women. Although trichomonads primarily infect the vagina, T. vaginalis can also be detected in endocervical swabs using NAAT. For diagnosis in men, NAAT appears to be more sensitive with urine than with urethral swabs. However, for NAAT detection of T. vaginalis in men, just as for culture, testing multiple specimens will substantially increase the number of cases identified. Highly sensitive and specific NAATs are particularly important for the diagnosis of T. vaginalis infection in men, since traditional microscopy and culture perform so poorly with specimens from men. In studies of men attending US STD clinics, T. vaginalis infection was detected by culture in 3–5% of men compared with 12–17% using PCR. In a study conducted in Malawi, in southeastern Africa, the addition of urethral swab PCR to wet-mount microscopy and urethral culture increased T. vaginalis detection from 16 to 21% in symptomatic men attending an STD clinic and from 9 to 12% in asymptomatic men attending a dermatology clinic compared with wet-mount and culture alone. Just as is the case for NAATs used to detect gonorrhoea and chlamydial infection, detection of T. vaginalis nucleic acid does not require the presence of viable organisms. This feature provides a distinct advantage when prolonged specimen storage and/or transport to the laboratory prohibit the use of T. vaginalis culture. In addition, the superb sensitivity of NAATs for a number of sexually transmitted pathogens allows the use of convenient, non-invasive specimens such as urine or self-obtained vaginal swabs, which may contain fewer organisms than semen or urethral swabs from men or clinician-collected vaginal swabs from women. However, consumers of NAAT results must also be mindful that these tests may remain positive for some time after treatment; although organisms have been killed, they may not be completely cleared from the genito-urinary tract immediately after treatment. Studies examining clearance of N. gonorrhoeae and C. trachomatis nucleic acids indicate that negative NAAT results can be expected one to two weeks following successful antimicrobial therapy. Similar kinetics are likely for T. vaginalis, but it will be important to establish the specific timeframe for clearance of trichomonad nucleic acids with newly developed diagnostic tests.

Improving Diagnosis and Management of Trichomoniasis
Perhaps the biggest obstacles to control of trichomoniasis are the lack of routine screening or testing for T. vaginalis infection in both male and female patients receiving care in various clinics, and the limited access to newer, more sensitive detection methods. In light of the high prevalence of trichomoniasis among HIV-negative and HIV-positive persons and the potential complications of untreated infections including pelvic inflammatory disease and adverse pregnancy outcomes, screening for T. vaginalis among asymptomatic at-risk patients should be strongly considered. Improved diagnosis of trichomoniasis involving infected patients and their sexual partners is needed as treatment with metronidazole 2gm or tinidazole 2gm orally in single doses is easy and highly effective. In a recent
study, over 70% of male partners of women with trichomoniass were infected with T. vaginalis, emphasising the importance of sexual partner notification and treatment.

Unfortunately, in many clinics and medical centres no diagnostic test beyond wet-mount microscopy is available. Although such testing can facilitate immediate diagnosis of infected women, clinicians should no longer consider wet-mount microscopy as an adequate diagnostic method for evaluation of T. vaginalis infection. Culture using the InPouch TV system is convenient and affordable (approximately US$3 per test device) and uses readily available technology. Rapid tests also provide a more sensitive immediate point-of-care diagnosis for trichomoniass and can be performed in clinics with limited laboratory capabilities. With the advent of the commercially available APTIMA TV test, clinicians in settings employing APTIMA NAATs to detect gonococcal and chlamydial infections can now obtain results for N. gonorrhoeae, C. trachomatis and T. vaginalis from a single specimen from men or women. Continued development and more widespread use of newer sensitive diagnostic tests, coupled with increased awareness of T. vaginalis as an important genito-urinary pathogen in men and women, are essential for control of trichomoniass.