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Trichomoniasis: a brief review of diagnostic methods and our experience with real-time PCR for detecting infection

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Abstract

Trichomoniasis is the most common non-viral sexually transmitted infection, and it is caused by the protozoan flagellate *Trichomonas vaginalis*. Although highly prevalent in sexually active women, it has long been overlooked in other groups of potentially infected people. Recently, studies have shown that trichomoniasis increases the risk of infection with human immunodeficiency virus and can cause adverse outcomes of pregnancy, which has increased interest in *T. vaginalis* and increased the need for highly sensitive diagnostic tests. This article summarizes the diagnostic methods most commonly used in the diagnosis of trichomoniasis, including the most sensitive and specific nucleic acid amplification tests. It also presents the results of our study comparing the performance of wet mount microscopy and culture to real-time PCR for detecting the parasite.

Keywords: review article, trichomoniasis, diagnosis, real-time PCR

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Introduction

Trichomoniasis is the most common non-viral sexually transmitted infection (STI), and it is caused by the protozoan flagellate *Trichomonas vaginalis*. Although highly prevalent, the disease is not reportable (1). In 2008, 276.4 million cases of *T. vaginalis* infection were estimated by the World Health Organization, ranking *T. vaginalis* incidence higher than that of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* combined (2). In contrast to other non-viral STIs, *T. vaginalis* infection rates increase with age (3, 4).

Humans are the only natural host of *T. vaginalis*. The parasite resides in the female lower genital tract and the male urethra and prostate. The infection is asymptomatic in 25 to 50% of infected women and in 70 to 80% of infected men (5, 6). In symptomatic individuals, a wide range of signs and symptoms are associated with the infection, similar to those caused by other STIs. In women, diffuse, malodorous, yellow to brown vaginal discharge can be present, which can be combined with vaginal itching and pain (vaginitis). Urethral infection is present in 90% of episodes, whereas lower abdominal pain is rare. Although not frequently identified, cervicitis characterized by petechial hemorrhages on the ectocervix ("strawberry cervix") may distinguish trichomoniasis from other causes of cervicitis. Trichomoniasis has been associated with pelvic inflammatory disease and adverse pregnancy outcomes, particularly premature rupture of membranes, preterm delivery, and low birth weight (7–9). In men, *T. vaginalis* can cause urethritis characterized by urethral discharge and dysuria, balanoposthitis, prostatitis, cystitis and epididymo-orchitis (5, 10). Several studies have shown that trichomoniasis doubles to triples the risk of acquiring human immunodeficiency virus (HIV) infection in women and increases sexual transmission of HIV (11–14).

Trichomonas vaginalis infection is often asymptomatic and, because they are similar to those of other STIs or diseases such as bacterial vaginosis, clinical manifestations are not reliable criteria for diagnosing trichomoniasis. Therefore, demonstration of the parasite is required for accurate diagnosis of the infection.

Laboratory diagnosis of trichomoniasis

Several tests are available in laboratory diagnosis of trichomoniasis, from basic microscopy to more complex rapid antigen and nucleic acid amplification tests (NAAT). The tests differ in their specificity and sensitivity, the complexity of their performance, and costs (7, 10). Diagnosis of *T. vaginalis* in men's specimens has been challenging given the lower parasite burden but seems promising with the NAAT breakthrough.

Microscopy

The traditional and most commonly used diagnostic method for trichomoniasis is microscopic examination of a wet mount preparation of vaginal or urethral secretions. The detection limit of microscopy is about 100 pear-shaped trichomonads with characteristic jerky or quivering motility per ml of specimen (10). The method is considered to be 100% specific but its sensitivity is poor, 44 to 68%, and is lower for specimens from men due to the lower parasite burden (7, 15, 16). Specimens should be examined within 10 to 20 minutes after collection to keep the parasite motile. The sensitivity of microscopy can be dramatically affected by delays between specimen collection and microscopic examination, as well as by suboptimal storage and transportation conditions of the specimen, especially by temperatures lower than 22 °C (7, 17).

Trichomonads can incidentally be found in conventional or liquid-based Papanicolaou (Pap) smears of cervical specimens, although none of them are currently in use for routine diagnosis of *T. vaginalis* (7, 18, 19).

Culture

Until recently, culture of the parasite in selective liquid media has been considered the gold standard for diagnosis of trichomoniasis. According to the recommendations of the Centers for Disease

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Control and Prevention (CDC), vaginal secretions should be cultured for *T. vaginalis* in each woman in whom trichomoniasis is suspected but not confirmed by microscopy (20). The sensitivity of culture is higher than that of microscopy, ranging from 44 to 95% (7, 10, 15, 16, 21). However, it is mandatory that specimens such as vaginal swabs and urethral swabs, urine, or semen from men be collected correctly, immediately inoculated into the medium (in less than 1 hour after collection), and properly incubated at 37 °C (22). Specimens can first be inoculated into transport systems to maintain viability of the parasite for up to 24 hours at room temperature, which is useful when immediate transportation of specimens to the diagnostic laboratory is not available. The most commonly used media for cultivating the parasite are Diamond's medium and the InPouch TV® culture system (BioMed Diagnostics, White City, OR, USA). InPouch TV® is a self-contained culture pouch that serves as the specimen's transport container, the growth chamber during incubation, and the slide during microscopy. Because it is made of optically clear plastic, once it is inoculated it requires no opening for microscopic examination. In contrast to Diamond's medium, which must be stored at 4 °C before use, InPouch TV® can be stored at room temperature. Once inoculated, it can remain at room temperature for up to 48 hours before incubation at 37 °C (7, 22). Cultures are to be examined microscopically each day for up to 5 days until proven negative (7).

Rapid diagnostic tests

The advantage of rapid diagnostic tests that detect *T. vaginalis* antigens or nucleic acids over microscopy and culture is that they are not limited by immediate transportation and rapid specimen processing. The OSOM Trichomonas rapid test® (Sekisui Diagnostics, Framingham, MA, USA) which is a US Food and Drug Administration (FDA)–cleared point-of-care antigen detection test, has been commercially available since 2003. It is an immunochromatographic capillary flow dipstick test that detects *T. vaginalis* membrane proteins in about 10 minutes. OSOM TV® is performed on vaginal secretions or swabs with 77 to 98% sensitivity and 99 to 100% specificity, but should not be used in asymptomatic women or men (7, 23–26). False positives might occur, especially in low-prevalence populations (20). Another commercially available, but not US FDA–cleared or European Union (EU) Conformité Européenne (CE)–marked, point-of-care antigen detection test is Tv latex® (Kalon Biological, Surrey, UK). It is a latex agglutination test with 55 to 99% sensitivity and 92 to 100% specificity (27, 28). The Affirm VPIII® (Becton Dickinson, Sparks, MD, USA) rapid diagnostic test is a nucleic acid probe hybridization test that evaluates for *T. vaginalis*, *Gardnerella vaginalis*, and *Candida albicans*. It is US FDA–cleared and bears the EU CE mark. The test uses specific oligonucleotide probes to detect unamplified nucleic acids of the microorganisms mentioned above. The performance of the test is much more complex than that of antigen detection tests. Its results are available within 45 minutes. The test has 46.3 to 64% sensitivity and 100% specificity (29, 30). It has not been evaluated for screening asymptomatic women or for diagnosing trichomoniasis in men (7).

Nucleic acid amplification tests

The development of highly sensitive and specific diagnostic tests based on amplification of *T. vaginalis* nucleic acid (e.g., polymerase chain reaction (PCR) and transcription-mediated amplification (TMA)) changed the view on diagnosis of trichomoniasis significantly. Because these tests are highly sensitive, they are suitable for screening (e.g., in epidemiological studies) and testing asymptomatic female and male patients. A variety of urogenital specimens can be used with NAATs, including urine, endocervical swabs, and self-collected vaginal swabs. As with rapid diagnostic tests, NAATs are not limited by immediate transportation at temperatures not lower than 22 °C and rapid specimen processing (7).

Among the commercially available NAATs, the TMA-based APTIMA assay® (Hologic Inc., San Diego, CA, USA) was the first to receive US FDA clearance and the EU CE mark for in vitro diagnostic (IVD) use to detect *T. vaginalis* in women's urine, endocervical and vaginal swabs, and endocervical specimens collected in specific solution (31). The test has 92 to 100% specificity and sensitivity and also performs well with specimens from men (16, 21, 23, 29, 32). The APTIMA assay® requires specific instrumentation and highly trained laboratory personnel, resulting in considerably higher costs (7).

In 2014, Cepheid announced the release of Xpert® TV (Cepheid, Sunnyvale, CA, USA), a qualitative real-time PCR test for automated, rapid, accurate, and reproducible detection of *T. vaginalis* in male and female samples. The test is marketed as an EU CE-IVD product and is the first nucleic acid amplification test to deliver trichomoniasis results for male urine samples (Cepheid TV Package Insert). The test offers sample to result in about an hour including sample preparation, with less than 1 minute of hands-on time. Xpert® TV is a kind of point-of-care test but requires a specific instrumentation.

Several in-house PCR tests for detecting *T. vaginalis* nucleic acid have been described in the international literature with sensitivity ranging from 84 to 100% and specificity 94 to 100% (16, 33–35). Because these tests are not commercially available, their validation is in the domain of the individual laboratory.

Our experience using real-time PCR for detecting *Trichomonas vaginalis* infection

From the beginning of February 2014 to the end of January 2015, a study was conducted at the Institute of Microbiology and Immunology (IMI), Faculty of Medicine, University of Ljubljana, to compare the performance of three methods for detecting *T. vaginalis* in urogenital swabs: wet mount microscopy, culture, and real-time PCR. Specimens were collected from 74 male and 76 female patients attending an outpatient department for STI at the Clinic for Infectious Diseases and Febrile Illnesses, Ljubljana University Medical Center, having either signs and symptoms of STI, sexual risk behavior, or a sex partner with confirmed STI. The average ages were 33.0 and 36.3 years for female and male patients, respectively. Out of 155 specimens collected from the 150 patients, 75 (48.4%) were urethral swabs and 80 (51.6%) were vaginal swabs.

The swabs were placed in CAT broth® medium (Copan, Brescia, Italy) immediately after collection and transported to IMI at room temperature in less than 2 hours. There, the medium with the swab was thoroughly agitated. The swab was discarded and the medium was centrifuged at 1,500 rpm for 5 minutes. A drop of the sediment was examined under the microscope for the presence of motile trichomonads, another part of the sediment was collected for detecting *T. vaginalis* by real-time PCR, and the rest was cultured at 37 °C. On each of the following 3 days, a drop of the incubated medium was examined microscopically for the presence of motile trichomonads.

Genomic DNA was extracted from 200 µl of the collected sediment using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). A TaqMan real-time PCR assay targeting a 92-bp fragment of the *T. vaginalis* specific repeat (36) was performed on the isolates using primers and a probe described by Pillay et al. (35). Real-time PCR was performed in a StepOne Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA).

Out of 155 specimens, all (100%) were negative by wet mount microscopy. After culture, 154 (99.4%) were negative and one (0.6%) vaginal swab was positive (Fig 1), whereas six specimens (five vaginal swabs from five female patients with an average age of 31.4 years and one urethral swab from a 31-year-old male patient) tested positive using real-time PCR resulting in 3.9% prevalence of trichomoniasis in the population studied. The results of testing positive patients are shown in Table 1.

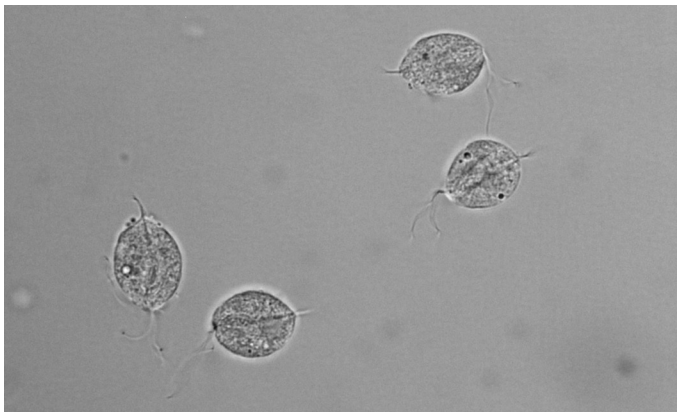

Figure 1 | *Trichomonas vaginalis* as seen by wet mount microscopy.

At the IMI, a routine diagnosis of trichomoniasis is currently based on wet-mount microscopy and culture. As has been shown previously, the main drawback of these tests is that they leave a large portion of infections undetected (15, 16, 21, 33, 35). The results of our study are in agreement with these findings. Given that the sensitivity of real-time PCR is superior to that of culture and wet-mount microscopy, its use for diagnosing trichomoniasis should be considered. However, because the test is considerably more expensive than wet-mount microscopy and culture and needs more hands-on-time, its modification into a multiplex real-time PCR for detecting multiple sexually transmitted pathogens would allow its wider application.

Table 1 | *Trichomonas vaginalis*–positive patients' data and the results of their testing by wet mount microscopy, culture, and real-time PCR.

Patient no.	Gender	Age (years)	Specimen	Wet mount microscopy	Culture	Real-time PCR
1	F	50	Vaginal swab	Neg	Pos	Pos
2	F	23	Vaginal swab	Neg	Neg	Pos
3	M	31	Urethral swab	Neg	Neg	Pos
4	F	26	Vaginal swab	Neg	Neg	Pos
5	F	30	Vaginal swab	Neg	Neg	Pos
6	F	28	Vaginal swab	Neg	Neg	Pos

Conclusion

Until recently, the public health impact of trichomoniasis was poorly understood. An application of highly sensitive tests to the laboratory diagnosis of *T. vaginalis* infection has revealed the true public health burden of symptomatic and asymptomatic *T. vaginalis* infections. Therefore the use of these tests in a routine diagnosis of trichomoniasis should be considered.

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