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Trichomonas vaginalis and challenges in laboratory diagnosis: A Narrative Review

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Abstract

Background: Trichomoniasis is considered one of the most common non-viral sexually transmitted diseases globally. Early diagnosis of *Trichomonas vaginalis* (*T. vaginalis*) infection plays an important role in effective treatment, infection control, and interruption of the transmission chain. The present narrative review was conducted to gather updated data and evaluate the challenges associated with available laboratory methods for the diagnosis of *T. vaginalis*.

Methods: A broad review of the published literature and electronic international databases, including ISI Web of Science, PubMed, Scopus, Science Direct, and Google Scholar, was conducted to identify relevant data on existing and newly developed diagnostic methods for trichomoniasis in both sexes. The search covered studies published from 2000 up to 2025. The benefits and limitations of these methods were compared.

Results: This review demonstrated that wet mount preparation and examination under a light microscope, accompanied by staining methods, are the most frequently used approaches worldwide. However, the sensitivity of this method is low. Direct microscopic examination accompanied by culture or staining provides a diagnostic strategy with good performance. Moreover, molecular- and immunological-based methods are recommended as complementary tests.

Conclusion: Direct microscopic examination is an economical and rapid method. Fully automated PCR systems for the simultaneous identification of the most common vaginal pathogens can be established as a diagnostic strategy in non-endemic areas and developed countries.

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Introduction

Trichomoniasis is one of the common sexually transmitted infections worldwide and is caused by the flagellated parasitic protozoan *Trichomonas vaginalis* (*T. vaginalis*) (1). Trichomoniasis is considered one of the most common non-viral sexually transmitted infections globally (2). The World Health Organization (WHO) estimated that there were 156.3 million new cases of trichomoniasis among individuals aged 15-49 years old (73.7 million in females, and 82.6 million in males) in 2020. WHO estimated that approximately one-third of new infections in this age group occur in the WHO African Region, followed by the WHO Region of the Americas (3). A systematic review and meta-analysis on the epidemiology of *T. vaginalis* infection estimated that the pooled mean prevalence of *T. vaginalis* was 4.7% (95% CI: 3.9-5.6%) among the resident female population in the Middle East and North Africa (4). A recent comprehensive study estimated a 4.30% prevalence of *T. vaginalis* among Iranian women (5).

Although many individuals infected with *T. vaginalis*, especially men, remain asymptomatic, trichomoniasis can present with clinical manifestations involving the urethral and vulvovaginal regions of the genital tract in both sexes. The most common signs and symptoms in infected women include edema, erythema, pruritus, dysuria, and atypical green, malodorous, and frothy vaginal discharge (6,7). Silent and chronic trichomoniasis may lead to complications such as prostate and cervical cancer, pelvic inflammatory disease, low birth weight in newborns, and premature birth (8,9). The association of *T. vaginalis* with increased acquisition and transmission of HIV/AIDS in populations where trichomoniasis is endemic has been well confirmed (10).

T. vaginalis, the causative agent of trichomoniasis, is a single-celled organism with only one morphological form, the trophozoite, and no known cyst stage. Trophozoites are typically pyriform and sometimes rounded, approximately 8-15 micrometers in size, and may reach 30 micrometers in length. They have four anterior flagella and one lateral flagellum attached to the undulating membrane (11). Early diagnosis and effective treatment of this parasitic disease play an important role in decreasing the disease transmission chain and preventing the consequences of infection. Traditional methods for the correct diagnosis of *T. vaginalis* in clinical samples are mainly based on microscopic analysis of wet smears and parasite cultures. Permanent stained smears for detecting the organism also exist and are used for identification of *T. vaginalis* in samples from infected individuals (12).

Currently, immunological-based assays and molecular methods are available and are used for the diagnosis of *T. vaginalis* infection. Each diagnostic method has different specificity and sensitivity. However, accurate diagnosis depends on appropriate specimen collection, the experience of laboratory personnel, examination time, and the type of diagnostic method used.

Various diagnostic methods are available for the detection of *T. vaginalis* infection. The present study aimed to gather updated data and review the various available diagnostic methods that have been used to detect and identify *T. vaginalis* in both female and male individuals. This review specifically focuses on recently developed diagnostic methods.

Methods

This investigation is a narrative review of existing and newly developed diagnostic methods for the identification of trichomoniasis. Electronic searches of international databases and published scientific journals were conducted to identify relevant data on diagnostic techniques for trichomoniasis in both sexes. The search covered articles published from 2000 up to 2025. Searches were performed in international databases, including Web of Science (ISI), the National Library of Medicine and the National Institutes of Health (PubMed), EMBASE, Scopus, Science Direct, and Google Scholar. To identify all related studies, a combination of relevant keywords and MeSH terms, including "Trichomoniasis OR *Trichomonas vaginalis*," "immunodiagnosis," "molecular," "serology OR serodiagnosis," "culture," "direct examination," and "diagnosis," was used as a keyword panel. The references of all related articles were checked to ensure the accuracy of the present search. The benefits and limitations of each method, such as sensitivity, specificity, time consumption, cost, need for trained personnel, and requirement for laboratory equipment, were compared.

Results

Direct microscopy examination

The traditional and routine method for diagnosing trichomoniasis is wet mount preparation of vaginal or urethral discharge and prostatic secretions, followed by microscopic examination. Vaginal discharge is ideally collected using a speculum and a cotton-tipped applicator. The specimen must then be diluted in a drop of saline. Wet mount examination is based on detecting trophozoites in samples according to their motility, presence of flagella, and other morphological characteristics, such as pear shape, jerky or tumbling motion, and the undulating membrane of the organism. Urine sediment and prostatic secretions can also be examined in the same manner as vaginal discharge. However, due to the low number of trophozoites in urethral secretions, direct examination of wet mount preparations from the male urethra is not recommended. Direct microscopic examination of sediment from the first-catch urine sample increases sensitivity; however, culture of urine sediment or the use of other more sensitive methods is preferable (13).

Direct microscopic examination is an economical and rapid method. This method is still frequently used in many diagnostic medical laboratories for diagnosing trichomoniasis. In this method, slides must be analyzed immediately after sample collection because the organisms lose motility within a few hours due to ex vivo conditions such as temperature differences (7,8). Under these conditions, trichomonad trophozoites retract their flagella, become rounder, lose motility, and change their morphology, thereby making them difficult to distinguish microscopically from similar cells, such as vaginal leukocytes. Furthermore, when a trophozoite is immobile, it can be challenging to differentiate it from vaginal epithelial cells, as its size may be similar to that of a lymphocyte or a small neutrophil.

The time dependence of sample examination and the experience of laboratory examiners are the main limitations of this technique. Brotman et al. reported that *T. vaginalis* trophozoites can survive for almost six hours in phosphate-buffered saline at room temperature, but their movement is significantly reduced (14). Thus, the sensitivity of direct microscopy has been reported to range from as low as 31% to as high as 82% in different studies compared with culture and molecular methods (13,15-17). A minimal concentration of 10 4 trophozoites of *T. vaginalis* per milliliter of vaginal discharge is necessary for detection of this organism using wet mount methods (18). To improve the sensitivity of this method, multiple specimens should be examined.

Staining

Permanent and non-permanent staining methods can be used in combination with wet mount preparation to increase the sensitivity of direct microscopy. Staining methods also allow specimens to be examined later when a large number of patients attend the gynecological outpatient department or medical laboratory and immediate wet mount microscopy is unavailable. In these methods, slides can be stored for educational purposes or referred to a specialist for further analysis. Different stains are used for the differential diagnosis of *Trichomonas* trophozoites from other cells. Techniques such as specific Giemsa staining (19), modified Field's staining (20), liquid-based Pap tests (21), safranin contrast stain, acridine orange staining, methyl violet staining (22), and calcofluor white staining (23) are used to identify *T. vaginalis* in vaginal smear preparations. *Trichomonas vaginalis* is routinely missed on Gram staining; therefore, this method is not used for trichomoniasis diagnosis.

A disadvantage of *T. vaginalis* staining methods is the loss of trophozoite motility during the fixation process, which makes parasite identification more difficult. In addition, the need for technical expertise and the time-consuming nature of staining are other disadvantages of these methods.

Giemsa staining is the most readily available method in medical diagnostic laboratories. It visualizes the internal and external structures of *Trichomonas*. In this method, the cytoplasmic zone stains blue, nuclear material stains red, and the undulating membrane and flagella are sharply visible (24). One limitation of using Giemsa staining is that it requires a heavy *T. vaginalis* infection because trophozoites may be lost or damaged during processing (24,25). The specificity and sensitivity of Giemsa and some other staining methods are compared in Table 1.

Acridine orange staining, which is used for fluorescence-based identification of *T. vaginalis*, is a non-specific staining procedure for nucleic acids (22). In acridine orange staining, *T. vaginalis* trophozoites appear brick red with a yellowish-green nuclear stain, while vaginal epithelial cells stain fluorescent light green. This staining method has high sensitivity among staining methods (71.4%-100%). However, it is not a permanent dye and requires specialized fluorescent microscopy (26). In safranin contrast staining, a drop of 0.1% safranin can stain leukocytes on a sample slide, while leaving *T. vaginalis* trophozoites unstained; therefore, they become conspicuous against a pink background. This staining method can be used instead of a direct wet mount when other stains are unavailable. The main limitation of safranin contrast staining is that it does not directly stain Trichomonas, similar to the wet mount method; therefore, organism identification still depends on the experience of the laboratory examiner in recognizing the morphology and motility of Trichomonas (23).

Calcofluor white staining is a method that uses fluorescent brighteners; calcofluor binds to cellulose and chitin on cyst-like structures of *T. vaginalis*, causing them to fluoresce under a UV light microscope. The cyst-like structures of this parasite are spherical, non-motile, and resistant forms of *T. vaginalis* that can form under certain conditions, such as prolonged culture or nutrient stress (27). It is believed that these cyst-like structures may play an important role in the transmission and pathogenesis of *T. vaginalis* (28). Further studies are needed to report the specificity and sensitivity values of Calcofluor White staining compared with other diagnostic methods for trichomoniasis.

Papanicolaou staining of a smear (Pap smear) is a rapid, simple, and inexpensive screening method mostly applied for the diagnosis of cervical neoplasms. Since a large number of women are screened by Pap smears annually worldwide, this technique can also be used for the laboratory identification of sexually transmitted diseases. Among staining methods, the sensitivity of the Papanicolaou test is low; therefore, it is not an ideal protocol for the diagnosis of *T. vaginalis* and is not recommended for trichomoniasis screening purposes (29,30). The sensitivity and specificity of Papanicolaou staining for diagnosis of *T. vaginalis* have been reported as 32% to 79% and 83% to 99%, respectively (31-34).

Modified Field's staining is a more rapid diagnostic staining method compared with traditional staining methods, such as the Giemsa technique, for identification of *T. vaginalis*. This method provides sharp morphological details of internal structures and requires only 20 seconds (20). However, the sensitivity and specificity of Modified Field's staining are unclear and need to be compared with other diagnostic methods for evaluation. It is suggested that staining techniques accompanied by wet mount examination of fresh samples be used to improve sensitivity in the laboratory diagnosis of *T. vaginalis* in routine medical diagnostic laboratories.

Culture

Culture of vaginal discharge, urine sediment, and prostatic secretions has been considered the gold standard method because of its high sensitivity in detecting positive cases of trichomoniasis (23). The sensitivity of this method has been reported between 75% and 97%, with a specificity of 100% (38-40). However, the culture method is time-consuming and requires viable organisms; therefore, it is not widely available in medical diagnostic laboratories (24). The incubation period for growth and identification of *T. vaginalis* in culture media ranges from two to seven days. Microscopic examination of cultures must be initiated after 24 hours of incubation and subsequently continued daily for up to seven days. A negative result is reported if no growth is observed after this period (13).

After sample collection, the viability of *Trichomonas* on a cotton swab smeared with vaginal secretions may be as short as 15 to 20 minutes (41). The specimen should be immediately inoculated into culture medium directly after collection. If a culture medium for cultivation of *T. vaginalis* is not available, a transport medium can preserve the viability of trophozoites before inoculation into culture medium for a limited time. The study by Beverly et al. showed that the viability of *T. vaginalis* in vaginal secretions can be maintained in Amies transport medium for up to 24 hours (42). The viability of *Trichomonas* in Stuart's transport medium, which is used as a transport medium for gonococci, extends beyond 24 hours (42).

T. vaginalis trophozoites are anaerobic protozoa that grow more rapidly under anaerobic conditions; therefore, use of a CO2 incubator is required for optimal growth and recovery of this parasite (43). The accuracy and ability of different culture media to detect *T. vaginalis* in clinical specimens are influenced by several factors, such as the nutritional content of the media, stability and longevity of the culture, sample contamination, the minimum number of organisms needed for inoculation, and incubation conditions (43,44).

Several liquid or broth culture media have been described for xenic and axenic cultivation of *T. vaginalis*, such as Kupferberg, Kupferberg STS, Hirsch, Robinson, Trichosel, Lash serum, Modified Diamond, and InPouch® TV. However, Diamond (TYM) and modified Diamond's TYI in glass tubes are the most commonly used culture media for the identification of *T. vaginalis* globally (7,38,39). Xenic culture of *T. vaginalis* is inexpensive and simple but requires direct microscopic examination of the media over an extended period. Axenic cultivation of *T. vaginalis* is expensive and has inherent limitations, such as contamination with bacteria or yeasts (Vaginal microbiota) (7,39).

Currently, several commercially available and ready-to-use media are available for the detection of *T. vaginalis*. A guideline proposed for the correct laboratory diagnosis of trichomoniasis recommends using culture media as a diagnostic tool for male patients, women with negative microscopy results for trichomoniasis but strong indications of vaginal infection, patients with severe or persistent signs and symptoms after completing specific drug therapy, and studies on *Trichomonas* resistance to antimicrobial agents (13).

A self-contained broth medium device for isolation of *T. vaginalis* from vaginal or urethral/urine samples is InPouch® TV medium (Biomed Diagnostics, Oregon, USA). The InPouch® TV medium consists of a double chamber separated by a single duct through which fluid can flow between chambers. The InPouch® medium contains essential components, such as peptones, maltose and other sugars, amino acids, and salts required for *Trichomonas* growth, along with antimicrobial agents in a phosphate-buffered saline base. InPouch® TV can be stored under room conditions for up to 48 h before incubation at 37°C (7,13,38). It has been shown that the InPouch® TV medium system is as sensitive as both modified Diamond's and Trichosel media (45,46). Two benefits of the InPouch® TV system are simultaneous sampling for microscopic examination and culture, as well as prevention of fungal and bacterial contamination in culture. The sensitivity of InPouch® TV is high and comparable to that of other culture media systems (46). Another advantage of the InPouch TV test is its relatively long shelf life (6 Months) at room temperature (13).

Cell culture systems are more sensitive than traditional media for cultivation of *T. vaginalis*. However, cell culture media are expensive and are rapidly contaminated by vaginal microbiota. Sample pretreatment with different antibiotics using an axenic culture medium, such as Diamond's TYI axenic medium, is required as a transfer and temporary medium before passage of the organism onto cell culture media. Cell culture enables the growth of *T. vaginalis* from a specimen containing as few as 3 organisms/mL (43,47). However, cell culture media have not been used for *Trichomonas* diagnostic purposes in medical diagnostic laboratories.

Immunological methods

Antigen detection

Antigen detection-based tests are important methods for easy and rapid diagnosis of trichomoniasis. These tests are based on immunochromatographic techniques that use specific monoclonal antibodies to detect *T. vaginalis* antigens. The benefit of this method is that no instrumentation for sample processing and testing is required. Results are typically available within 10-15 minutes; therefore, these tests are widely used in point-of-care settings. Moreover, these tests can be performed in emergency rooms, gynecological clinics, and self-test home programs. Due to their high sensitivity and specificity, antigen detection tests can also be used in epidemiological studies or as part of high-volume medical laboratory analyses (21). Several commercially available antigen detection kits are available for identifying trichomoniasis, including the OSOM rapid test for *Trichomonas* (Sekisui Diagnostics, California, USA), the *T. vaginalis* latex agglutination test (Kalon Biological, Surrey, UK), and XenoStrip-TvTM (Xenotope Diagnostics, Inc., USA) (1,21,48). It has been shown that the OSOM rapid test for *Trichomonas* is highly acceptable as a home test, with 82-95% sensitivity and 97-100% specificity (49-51). However, its sensitivity of 37.5% and specificity of 82.9% have been reported for detecting *T. vaginalis* in men (52). This test can also be used for diagnosis and follow-up of treatment of vaginal protozoan infection in the emergency department (49). OSOM is a qualitative test that uses immunochromatographic capillary flow enzyme immunoassay to identify a specific *Trichomonas* antigen, known as alpha-actinin protein. OSOM has high reliability and provides results in approximately ten minutes (1).

The latex agglutination test for *T. vaginalis* (Kalon) is a kit that uses a suspension containing latex beads coated with specific antibodies (Rabbit anti-*T. vaginalis* IgG) to detect *Trichomonas* protein antigens. In this slide test, *T. vaginalis* antigen present in the diluted sample of vaginal exudate causes agglutination of the sensitized latex. *T. vaginalis* antigens mixed with latex beads agglutinate on a glass slide when they are present in the eluate from a vaginal swab. No equipment is required, and sample processing and examination results are available in less than ten minutes (53). The sensitivity of agglutination tests is comparable to that of culture, but they cannot be used for male patients. Yousofi-Daran et al. developed an agglutination kit that simultaneously identifies trichomoniasis and vaginal candidiasis (54).

XenoStrip-TV is a rapid qualitative test based on color immunochromatographic "dipstick" technology for identifying the specific antigenic protein alpha-actinin in swabs of vaginal specimens (55). The results of this test are read at 10 min. Pillay et al. reported 52.6-78.9% sensitivity for the XenoStrip-TV test compared with molecular testing for identification of *T. vaginalis* infection. However, the XenoStrip-TV test is significantly more sensitive than the direct microscopy method, which is routinely used in medical laboratories and clinics worldwide (55). The specificity of this test is 100%, and it did not result in any false positives compared with the gold standard method. In another small evaluation study, 90% sensitivity and 92.5% specificity were reported for the XenoStrip-TV method compared with InPouch TV culture (56).

Although antigen detection kits for trichomoniasis offer several advantages, this method has been reported to have lower sensitivity in men and asymptomatic individuals compared with molecular-based methods; therefore, these tests are primarily recommended for use with vaginal specimens from symptomatic women (57).

Serological tests (Antibody detection):

Due to the localized infection of *T. vaginalis* in the human urogenital tract, trichomoniasis does not typically elicit a strong systemic immune response in most cases. It is currently well known that antibody detection tests are more frequently positive in symptomatic patients than in asymptomatic patients (12). It is estimated that there are eight serotypes, and numerous antigenic markers of *T. vaginalis* have been shown in immunoblot experimental studies (43). The serological response to trichomoniasis according to distinct parasite serotypes may vary among different individuals who react to various parasite-specific antigens. Several methods, such as complement fixation test, gel diffusion, fluorescent antibody tests, hemagglutination, and ELISA, have been used for *Trichomonas* antibody detection (43). Indirect ELISA has been used in numerous scientific studies to detect specific antibodies against trichomoniasis because of its sensitivity and specificity (7,58-60). The epitopes used in the ELISA method and recognized by the patient's antibodies must be completely specific to *T. vaginalis* to avoid cross-reactions and false positives. Some antigens of *T. vaginalis*, such as alpha-actinin, alpha-enolase, aldolase, and glyceraldehyde-3-phosphate dehydrogenase, stand out for their immunogenicity (7,61). To increase the specificity and sensitivity of the ELISA test, obtaining synthetic recombinant peptides that contain multiple epitopes and can be identified by a large number of specific antibodies has been proposed (61).

In the absence of recombinant antigen technology, the use of a lysate of various serotypes or strains of *T. vaginalis* is recommended to perform an indirect ELISA and quantify the seropositivity of suspected individuals. A sensitivity of 88.9% and specificity of 97.1% compared with the culture method have been reported for the sandwich ELISA technique when specific capture antibodies immobilized on the micro titer plate are used for diagnosis of specific *T. vaginalis* proteins (62). Immunofluorescence techniques are not routinely employed for the diagnosis of trichomoniasis because they require a fluorescence

microscope and trained personnel. However, they may be useful assays for confirming negative results from wet mount or culture examination methods.

Patel et al. systematic review estimated 82% (74-90%) sensitivity of the enzyme-linked immunosorbent assay and 73% (35-100%) specificity compared with molecular and culture methods. They reported 85% sensitivity (79-90%) and 99% (98-100%) specificity for the direct fluorescence antibody test (63). A comparison of the specificity and sensitivity of different methods for *T. vaginalis* detection is shown in Table 2. However, these serological methods are not specific for differentiating between past and recent infections. They are also sophisticated methods that require trained personnel and laboratory equipment, which may not be available in many routine medical diagnostic laboratories.

Molecular methods

Molecular methods have been shown to be the most sensitive methods for the diagnosis of *T. vaginalis*. Depending on the target sequence and type of primers employed, the sensitivity of molecular methods typically ranges from 85% to 100% (20,64-66), and specificity has also been reported as 95-100% (16,67). Molecular methods do not require live organisms and can be used for different clinical specimens. The high sensitivity of molecular methods makes them suitable for testing asymptomatic men and women, as well as for screening and epidemiological studies. However, these tests are not suitable for routine diagnostic medical laboratories due to limitations such as high cost, time consumption, the need for trained laboratory personnel, and the requirement for specialized equipment.

Different urogenital specimens from men and women can be used for molecular methods, including urine sediment, self-collected or clinician-collected swabs, endocervical and urethral swabs, as well as endocervical samples collected for liquid-based Pap smears (21). One benefit of some molecular-based methods, such as multiplex or fully automated real-time multiplexing PCR systems that have recently been developed, is the ability to readily detect and simultaneously differentiate *T. vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Candida* as primary vaginal pathogens in either endocervical or vaginal specimens with high sensitivity (68). Currently, several companies have developed commercial diagnostic kits for the differential detection of multiple urogenital pathogens. For example, Xpert® *Trichomonas vaginalis*, BD CTGCTV2, and Alinity m STI are among these kits (7). Recently, a multiplex RT-PCR assay has also been developed for the simultaneous diagnosis of nine urogenital pathogens, including *T. vaginalis*, *C. trachomatis*, *N. gonorrhoeae*, *C. albicans*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *M. genitalium*, *Ureaplasma urealyticum*, *U. parvum*, and human herpes viruses, with 91.06-100% sensitivity and 99.14-100% specificity (68). High sensitivity and specificity have also been reported for loop-mediated isothermal amplification (LAMP) assays for the diagnosis of *T. vaginalis* in urine and genital samples (69). However, it should be noted that molecular methods, especially automated simultaneous diagnostic systems, are mostly used in medical diagnostic laboratories in developed countries or in research laboratories. They have not yet been established as routine and economical methods in medical diagnostic laboratories in developing countries.

Table 1. Comparison of sensitivity and specificity of staining methods in diagnosis of *T. Vaginalis*

Staining method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Comparison method	References
Giemsa	52-80	40-98.3	40-66.7	66.6-99.1	Wet mount	22,26
	66.67	98.96	80	97.94	PCR	24
	63.6-85.7	97.4-100	75-100	69.2-98.7	Culture	35,36
Acridine orange	71.4-100	71.4-99.4	50-67.6	100	Wet mount	22,26
	73.5-81.8	99.6-100	95.2-100	97.8-98.9	Culture	35,37
Papanicolaou	32.6-100	68.9-98.9	33.3-93.3	75.6-100	Culture	30,34
	98	96	88	99	Wet mount	33

PCR: Polymerase Chain Reaction

Table 2. Comparison of sensitivity and specificity of different diagnostic methods of trichomoniasis

Diagnostic test	Technique	Sensitivity %	Specificity %	References
Microscopy	Wet mount	31-82	up to 100	13,15-17
Culture	Xenic and axenic culture	75-97	100	38-40
Staining	Acridine orange, Giemsa, Papanicolaou	32.6-100	66.7-100	20,22,26,33
Rapid antigen detection	OSOM, Kalon XenoStrip-Tv	52-95	97-100	49,55,70
Antibody detection	Hemagglutination, Immunofluorescent, ELISA	66-100	35-100	26,59,62,63
Nucleic acid amplification tests	PCR, Multiplex, real-time PCR, RFLP, automated molecular systems, LAMP	85-100	95-100	13,16,64-67

PCR: Polymerase Chain Reaction

OSOM: OSOM Trichomonas rapid test

ELISA: Enzyme-Linked Immunoassay

RFLP: Restriction Fragment Length Polymorphism

LAMP: Loop-mediated Isothermal Amplification

Discussion

Trichomoniasis is considered one of the most common non-viral sexually transmitted diseases globally (2). Laboratory diagnosis of trichomoniasis can be challenging because of issues related to the selection and accuracy of testing methods. For diagnosis of both symptomatic and asymptomatic *T. vaginalis* infections, wet mount preparation from vaginal or urethral discharge and prostatic secretions, followed by examination under a light microscope and accompanied by staining methods, is among the most frequently used approaches in medical laboratories worldwide. However, the sensitivity of wet mount and staining methods has been reported to be low (15-17). Culture methods are reported as the gold standard for laboratory diagnosis of trichomoniasis and are sometimes essential for accurate diagnosis. Several liquid or broth culture media have been described for the cultivation of *T. vaginalis*. One of the best culture media is the InPouch® TV system (46). The benefit of the InPouch® TV system is simultaneous sampling for wet mount microscopic examination and culture. The highest sensitivity in identifying positive cases of trichomoniasis has been reported using culture media (23). However, culture techniques are time-consuming.

New diagnostic methods for detecting trichomoniasis, such as nucleic acid amplification tests and rapid specific antigen detection, have recently become commercially available. These methods do not require the presence of viable *T. vaginalis* trophozoites in specimens (30,49,69,70). Therefore, it is important to compare sensitivity, specificity, time consumption, the need for trained laboratory personnel, and economic performance to select a routine detection method for *T. vaginalis* infection in diagnostic medical laboratory settings. The high specificity and sensitivity of rapid specific antigen detection and molecular methods make them suitable for testing, especially in asymptomatic infected men and women. However, these tests are not suitable or widely applied in routine diagnostic medical laboratories in developing countries.

Conclusion

For laboratory diagnosis of trichomoniasis, direct microscopic examination accompanied by culture or staining is a diagnostic strategy with good performance for many medical diagnostic laboratories worldwide. Moreover, molecular- and immunological-based methods are recommended as complementary tests. Fully automated PCR systems for the simultaneous identification of the most common vaginal pathogens can be established as a diagnostic strategy in medical diagnostic laboratories in non-endemic areas and developed countries.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Hossein Hooshyar. Data curation: Hossein Hooshyar, Parvin Rostamkhani. Investigation: Hossein Hooshyar, Parvin Rostamkhani, Mohsen Arbabi. Methodology: Hossein Hooshyar, Mohsen Arbabi. Resources: Hossein Hooshyar. Software: Mohsen Arbabi. Supervision: Hossein Hooshyar. Validation: Hossein Hooshyar. Writing-original draft: Hossein Hooshyar, Parvin Rostamkhani. Writing-review and editing: Hossein Hooshyar, Parvin Rostamkhani, Mohsen Arbabi.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence

The authors declare that no artificial intelligence (AI) tools or services were used in the preparation of this manuscript.

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