Trichomonas vaginalis and challenges to laboratory diagnosis: A Narrative Review

Running title: *Trichomonas vaginalis* laboratory diagnosis

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Abstract

Background: Trichomoniasis is considered as the most common non-viral sexually transmitted diseases globally. Early diagnosis of *Trichomonas vaginalis* (*T. vaginalis*) infection has an important role in effective treatment and controlling the infection and interrupt of transmission chain. The present narrative review was carried out to gather new data and evaluate the challenges of available methods of laboratory of *T. vaginalis* diagnosis.

Methods: With a broad review of the published literature and electronic international databases such as ISI Web of Science, PubMed, Scopus, Science Direct, and Google Scholar was conducted to find relevant data reporting on existing and newly developed diagnostic methods for the identification of trichomoniasis diagnostic techniques in both sexes and covered 2000 up to 2025. The benefit and limitation of these methods were compared.

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

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

Key word: *Trichomonas* infection, *Trichomonas vaginalis,* Diagnosis, Laboratories

Introduction

One of the common sexually transmitted infections caused by the flagellated parasitic protozoan *Trichomonas vaginalis* (*T. vaginalis*) is trichomoniasis worldwide (1). Trichomoniasis is considered one of the most common non-viral sexually transmitted infections globally (2). It is 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) by the World Health Organization (WHO) in 2020. WHO is estimated that approximately one- third of new infections occur in the WHO African Region in this age group, followed by the WHO Region of the Americas (3). A systematic review and meta-analysis study on the epidemiology of *T. vaginalis* infection estimated that the pooled mean prevalence of *T. Vaginalis* is 4.7% (95% CI: 3.9-5.6%) in the resident population of women in the Middle East and North Africa (4). A recent comprehensive study estimated a 4.30% prevalence of *T. vaginalis* in Iranian women (5).

Although many individuals, especially men infected with *T. vaginalis* remain asymptomatic, trichomoniasis can develop clinical manifestations in the urethral areas of the genital tract and vulvar in both sexes. The most common signs and symptoms in infected women are edema, erythema, pruritus, dysuria, and an atypical green, malodorous, and frothy discharge of vagina (6-7). The silent and chronic trichomoniasis may be leading to some difficulties such as prostate and cervical cancer, pelvic inflammatory disease, and low birth weight in newborns and premature birth (8-9). The relevance of *T. vaginalis* with increased acquisition and transmission of HIV/AIDS in populations where trichomoniasis is endemic is well confirmed (10).

T. vaginalis, the causative agent of trichomoniasis, is a single- celled organism with only one morphological form, the trophozoite, without a 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 adjust 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 correct diagnosis of *T. vaginalis* in clinical samples are mainly based on microscopic analysis of wet smears and parasite cultures. Permanent stained smears for finding the organism also exist and are used for identification of *T. vaginalis* in samples of infected individuals (12).

Nowadays, immunological-based assays and molecular methods are available and are used for the diagnosis of *T. vaginalis* infection. Each diagnostic method shows different specificity and sensitivity. However, accurate diagnosis depends on the appropriate specimen collection, the experience of laboratory personnel, examination time, and the type of diagnostic methods used. Various diagnostic methods are considered for the detection of *T. vaginalis* infection. The present study aims to gather new data and review the available various diagnostic methods that have been used to detect and identify of *T. vaginalis* in both female and male individuals. This review, specially focuses on review the new diagnostic methods that have recently been developed.

Methods

This investigation is a narrative review of existing and newly developed diagnostic methods for the identification of trichomoniasis. Electronic searches in electronic international databases and published scientific journals were conducted to find relevant reported data on diagnostic techniques for identifying of trichomoniasis in both sexes. The search covered the articles published in 2000 up to 2025. Searching was performed in the international databases covering 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 to identify all related studies, as a panel of keywords. All references of related articles were checked to ensure the accuracy of present search. All benefit and limitation of each method such as sensitivity, specificity, time-consuming, cost, require to trained personnel and laboratory equipment were compared.

Result

Direct microscopy examination

The traditional and routine method for diagnosing trichomoniasis is preparation wet mount of vaginal or urethral discharge and secretions of prostate followed by examination under a microscope. Vaginal discharge is ideally collected using a speculum on a cotton-tipped applicator. The specimen must then be diluted in a drop of saline. The wet mount examination is based on finding trophozoites in the samples based on their motility, presence of flagella, and other morphological characteristics such as their pear shape, jerky or tumbling motions, 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 microscopy examination of sediment from the first-catch urine sample increases sensitivity; however, culture of the urine sediment or using other more sensitive methods is ideal (13).

Direct microscopy examination is an economical and rapid method. This method is still frequently used in many diagnostic medical laboratories for diagnosis trichomoniasis. In this method the 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). In this condition, trichomonad trophozoites, retract their flagella, become rounder, loss motility and change their morphology; thereafter, making them difficult to distinguish microscopically from the similar cells such as and vaginal leucocytes. Furthermore, when a trophozoite is immobile, it can be challenging to differentiate from vaginal epithelial cells, as their size will be similar to that of lymphocyte or a small neutrophil cell.

Time dependence of sample examination and the experience of laboratory examiners persons 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 significant reduced (14). So, the sensitivity of direct microscopy method is reported as low as 31% to as high as 82% in different studies in comparison 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 as a low amount of *Trichomonas* for the detection of this organism using wet mount methods (18). To improve the sensitivity of this method, multiple specimens must 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 the examination of a specimen later, when a large number of patients are attending the gynecological outpatient department or medical laboratory and immediate microscopy examination of a wet mount is unavailable. In these methods, slides can be stored for educational purposes or to be referred to a specialist for further analysis. Different stains are used for the differential diagnosis of *Trichomonas* trophozoites from other cells. Technique 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 preparation smears. *Trichomonas vaginalis* routinely missed on Gram staining and is not used for trichomoniasis diagnosis.

The disadvantage of *T. vaginalis* staining methods is the loss of motility of trophozoites during the fixation process, which leads to difficulty in parasite identification. In addition, requiring technical expertise and being time-consuming are other disadvantages of staining methods.

Giemsa staining is the most readily available method in the medical diagnostic laboratories. It visualizes the internal and external structures of *Trichomonas*. In this method the cytoplasmic zone stains blue, the nucleus materials stains red, and the undulating membrane and flagella are sharply seen (24). One limitation for using of Giemsa staining is that it requires a heavy *T. vaginalis* infection due to the loss or damage of trophozoites 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 acid (22). In acridine orange staining, trophozoites of *T. vaginalis* appear brick red with a yellowish-green stain of nucleus, while vaginal epithelial cells stained fluoresce light green. This method of staining has a high sensitivity among stanning methods (71.4%-100%). However, it is not a permanent dye and requires specialized fluorescent microscopy (26). In safranin contrast stain, a drop of 0.1% safranin can stain leukocytes on a slide of a sample, whereas leaving *T. vaginalis* trophozoite unstained, it and will be 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 stain is that it does not directly stain the trichomonas, such as the wet mount method, so identification of the organism is still dependent on the experience of laboratory examiner to recognize the morphology and motility of trichomonas (23).

Calcofluor white staining is a method that uses fluorescent brighteners that calcofluor binds to cellulose and chitin on cyst-like structures of *T. vaginalis*, causing them to fluorescence 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 have an important role in the transmission and pathogenesis of *T. vaginalis* (28). Further studies are needed to reporting the specificity and sensitivity values of Calcofluor White staining compared to other diagnostic methods for trichomoniasis.

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

Modified Field's staining is a more rapid diagnostic staining method compare to traditional staining methods such as the Giemsa technique for identification of *T. vaginalis.* This method provides sharp morphological details of internal structures and only takes 20 seconds (20). However, the sensitivity and specificity of Modified Field's staining method are not clear 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 in order to improvement the sensitivity in the laboratory diagnosing of *T. vaginalis* in routine medical diagnostic laboratories.

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 98	68.9-98.9 96	33.3-93.3 88	75.6-100 99	Culture Wet mount	30,34 33

PCR: Polymerase Chain Reaction

Culture

The culture of vaginal discharge, urine sediment, and prostatic secretions was intended the gold standard method due to its highest sensitivity in detecting positive cases of trichomoniasis (23). The sensitivity of this method is reported between 75% and 97% with a specificity of 100% (38-40). However, the culture method is time-consuming and needing viable organisms, so it is not widely available in medical diagnostic laboratories (24). The incubation periods times for growth and identification of *T. vaginalis* in culture media rang from two to seven days. Microscopic examination of cultures must be initiated after 24 hours of incubation and subsequently continue daily for up to seven days. A negative result was reported if no growth is seen after this period (13).

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

T. vaginalis trophozoite is an anaerobic protozoan that more rapidly grows in anaerobic situation, so using a CO_2 incubator has been needed for optimal growth and recovery of this parasite (43).The accuracy and ability of the different culture media in detecting *T. vaginalis* in clinical specimens are influenced by several factors, such as the nutritional content of the media, the stability and longevity of the culture, sample contamination, the minimum number of organisms needed for inoculation, and the 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 culture mediums used for the identification of *T. vaginalis* globally (7, 38-39). The xenic culture of *T. vaginalis* is inexpensive, and simple but need to the direct microscopic examination of media for 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).

Nowadays, there are some commercially available and ready-to-use media available for use in 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 result in microscopy exam for trichomoniasis but with a strong indication of vaginal infection, patients with severe or continuing signs and symptoms of disease after completing specific drug therapy, and for study on *Trichomonas* resistance to antimicrobial agents (13).

A self-contained broth media device for isolation of *T. vaginalis* from vaginal or urethra/urine samples is InPouch® TV medium (Biomed Diagnostics, Oregon, USA). The InPouch® TV medium consists of a doubled chamber, which are separated by a single duct that fluid can flow between chambers. The InPouch® medium contains some necessary elements such as peptones, maltose and other sugars, amino acids, and salts that are needed for the growth of *Trichomonas* along with antimicrobial agents in a phosphate-buffered saline base. InPouch® TV can be stored at room conditions for up to 48 h prior to incubate at 37°C (7, 13, 38). It has been showed that the InPouch® TV medium system is as sensitive as both modified Diamond's and Trichosel mediums (45-46). Two benefits of the InPouch® TV system is simultaneous sampling for microscopy examination and culturing in addition to preventing the culture method from being contaminated by fungi and bacteria. The sensitivity of InPouch® TV is high and comparable to other culture media systems (46). Another advantage of the InPouch TV test is a relatively long-term shelf life (6 months) at room condition (13).

Cell culture systems are more sensitive than traditional medium for cultivation of *T. vaginalis.* However, cell culture media are expensive and rapidly contaminated by vaginal microbiota. Sample pretreatment with different antibiotics using an axenic culture medium such as Diamond's TYI axenic medium is needed as a transfer and temporal medium before passage organism onto the cell cultures 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 has not been used for *Trichomonas* diagnosis proposes in medical diagnostic laboratories.

Immunological methods

Antigen detection

Antigen detection-based tests are an important method for easy and rapid diagnosis of trichomoniasis. These tests are based on immunochromatographic techniques that uses 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 prepared within 10–15 minutes, so they 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 high sensitivity and specificity, these antigen detection tests can also be used in epidemiological studies or as part of high-volume medical laboratory analysis (21). There are several commercially available antigen detection kits 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-Tv^{TM} (Xenotope Diagnostics, Inc., USA) (1, 21, 48). It is 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% 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 utilizes immunochromatographic capillary flow enzyme immunoassay to identify of specific *Trichomonas* antigen, which is named α-actinin protein. OSOM has high reliability and is prepared in approximately ten minutes (1).

The latex agglutination test for *T. Vaginalis* (Kalon) is a kit that uses a suspension containing latex beads that are coated with specific antibodies (rabbit anti-T. vaginalis IgG) to detect *Trichomonas* protein antigens. In this slide test, the antigen of *T. vaginalis* present in the diluted sample of vaginal exudate causes agglutination of the sensitized latex. The antigens of *T. vaginalis* mixed with the latex beads agglutinate on a glass slide when they are present in the eluate from a vaginal swab. No equipment is needed, and sample processing and examination results are prepared in less than ten minutes (53). The sensitivity of agglutination tests is comparable to culture, but it 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 identify the specific antigenic protein alpha-actinin in swab of vaginal specimens (55). The results of this test were read at 10 min. Pillay et al. reported a 52.6-78.9% sensitivity for the Xenostrip-TV test compared to molecular test for identify of *T. vaginalis* infection. However, 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 not resulted to any false positives compared to the gold standard method. In another small evaluation study, a 90% sensitivity and 92.5%specificity were reported for the Xenostrip-TV method in comparison to InPouch TV culture (56).
Although antigen detection kits for trichomoniasis offer several advantages, it is reported that this method commonly has less sensitivity in men and asymptomatic individuals compared to molecular-based methods, therefor these tests are primarily recommended for use with vaginal specimens from symptomatic women (57).

Serological tests (Antibody detection):

Due to localized infection of *T. vaginalis* in the human urogenital tract, trichomoniasis dose 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 compared to asymptomatic patients (12). It is estimated that there are eight serotypes, and a numerous of antigenic markers of *T. vaginalis* have been shown in immunoblot experimental studies (43). The serological response to trichomoniasis according to distinct parasite serotypes may be changeable among different individuals who react to various parasitic specific antigens. Some variety of methods, such as complement fixation test, gel diffusion, fluorescent antibodies, hemagglutination, and ELISA, have been used for *Trichomonas* antibody detection (43). The indirect ELISA has been used in numerous scientific studies for detecting specific antibodies against trichomoniasis due to its sensitivity and specificity (7, 58-60). The epitopes used in the ELISA method that are used 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 α-actinin, α-enolase, aldolase, and glyceraldehyde-3-phosphate dehydrogenase stand out for their immunogenicity (7, 61). For the purpose of increase the specificity and sensitivity of the ELISA test, obtain the synthetic recombinant peptides, which contain multiple epitopes which can be identified by an excess number of specific antibodies, is proposed (61).
In the absence of recombinant antigen technology, use a lysate of various serotypes or strains of *T. vaginalis* recommended to perform an indirect ELISA and quantify the seropositivity of suspected individuals. An 88.9% sensitivity and 97.1% specificity compared to the culture method is reported for the sandwich ELISA technique when using specific capture antibodies immobilized on the microtiter plate for diagnosis of specific *T. vaginalis* proteins (62). Immunofluorescence techniques are not employed for the diagnosis of trichomoniasis routinely due to the need for fluorescence microscope and trained personnel. However, it could be a benefit assay for confirming of negative results of wet mount or culture examination methods.

Patel et al. systematic review estimated an 82% (74-90%) sensitivity of the enzyme-linked immunosorbent assay, and 73% (35-100%) specificity compared to 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 in *T. vaginalis* detection is shown in Table 2. However, these serological methods are certainly not specific in differentiating between past and recent infections. Also, it is a sophisticated method and require trained personnel, and laboratory equipment that may be not available in many routine medical diagnostic laboratories.

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, Geimsa, 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

Molecular methods

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

Different urogenital specimens from men and women can be used for molecular methods, including urine sediment and self or clinically collected sample swabs, endocervical and urethral swabs; in addition, 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 differential simultaneous diagnose of *T. vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Candida* as primary vaginal pathogens in either endocervical or vaginal

specimens with high sensitivity (68). Nowadays, several companies have developed some commercial diagnostic kits for the differential detection of multiple urogenital pathogens. For example, Xpert® *Trichomonas vaginalis*, BD CTGCTV2, and Alinity m STI are some of these kits (7). Recently, a multiplex RT-PCR has also been developed for the simultaneously 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 a 91.06-100%sensitivity and 99.14-100%specificity (68). Also, a high sensitivity and specificity were 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 are not yet established as routine and economical methods in medical diagnostic laboratories in developing countries.

Discussion

Trichomoniasis is considered as the most common non-viral sexually transmitted diseases globally (2). Trichomoniasis can be challenging to diagnose in the laboratory due to the problems associated with selection and accuracy of the testing method. To diagnose both symptomatic and asymptomatic *T. vaginalis* infections, wet mount preparation from vaginal or urethral discharge and prostatic secretions and examination under a light microscope accompanied by staining methods are the most frequently used in medical laboratories worldwide. However, the sensitivity of wet mount and staining methods are reported low (15-17). The culture methods are reported as gold standard for the trichomoniasis laboratory diagnosis and sometimes are essential for the accurate diagnosis of the trichomoniasis. Several liquid or broth culture media have been described for cultivation of *T. vaginalis*. One of the best culture media is InPouch® TV system (46). The benefit for the InPouch® TV system is simultaneous sampling for wet mount microscopy examination and culture. The highest sensitivity in identification of positive cases of Trichomoniasis 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, recently became commercially available. These methods do not need to presence of viable *T. vaginalis* trophozoites in specimens (30,49, 69-70). It is therefore of interest and important to compare the sensitivity and specificity, time consumption, need for trained laboratory personnel and economic performance to select a routine detection method for *T. vaginalis* infection in diagnostic medical laboratories settings. The high specificity and sensitivity of rapid detection of specific antigens and molecular methods make them suitable for testing, especially in asymptomatic infected men and women. However, these tests are not suitable and applied in routine diagnostic medical laboratories in developing countries.

Conclusion

For laboratory diagnosis of trichomoniasis, direct microscopy examination accompanied by culturing, or staining, is a good-performance diagnostic strategy for many medical diagnostic laboratories worldwide. Moreover, molecular- and immunological-based methods are recommended to be used as complementary tests. The 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.

Author Contribution

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