Microscopy Diagnosis of Trichomoniasis

Subjects: Infectious Diseases

Contributor: Alexandra Ibáñez-Escribano , Juan José Nogal-Ruiz

More than one million curable sexually transmitted infections occur every day. *Trichomonas vaginalis* is one of the main infections responsible for these epidemiological data. The diagnosis of this protozoan is mainly based on microscopic and culture identification.

trichomoniasis sexually transmitted infections diagnosis microscopy

1. Introduction

Sexually transmitted infections (STIs) remain a major public health concern. More than 30 pathogens can be transmitted through sexual intercourse, and nearly one million people are infected with a curable sexually transmitted pathogen every day [1][2]. The World Health Organization estimated an incidence of more than 377 million cases of chlamydia, gonorrhea, trichomoniasis, and syphilis in women and men during 2020 [3]. Although the incident cases of trichomoniasis are nearly 156 million [4], these epidemiological data may be underestimated due to the high number of asymptomatic patients [5], the low sensitivity of the preferred diagnostic methods used in many regions [6], and the fact that *Trichomonas vaginalis* infection is not a notifiable disease [7]. For all these, trichomoniasis has been included in the list of neglected parasitic infections (NPI) by the Center for Disease Control and Prevention (CDC) [8].

Trichomoniasis is characterized by a wide range of signs and symptoms associated with the inflammatory response triggered by the settlement of the parasite [9]. In women, about 75% of patients develop clinical manifestations. The most common are pruritus, local edema, erythema, dysuria, and/or a typical green, frothy, and malodorous vaginal discharge, among others [5][10][11]. In men, nearly 80% are asymptomatic; however, nongonococcal urethritis, epididymitis, or prostatitis may occur [5][10]. Although trichomoniasis has been considered as a “nuisance” infection [12][13], the complications and risks associated with this STI have led to its inclusion in the WHO Global Health Strategy on STIs for the period 2022–2030 [2]. *T. vaginalis* increases, by 1.5 times, the risk of acquiring HIV [14] but also favors its transmission due to the imbalance in the vaginal microbiome, the proinflammatory immune response, and the elevated vaginal pH [15]. In this scenario, coinfections with different urogenital pathogens are common among women with trichomoniasis, i.e., *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, human papillomavirus, or herpes simplex virus types 1 and 2 [16][17]. Other serious sequelae include pelvic inflammatory disease (PID) [2], adverse pregnancy outcomes [9][18], and infertility [9]. Moreover, several studies have associated trichomoniasis with an increased risk of cervical carcinogenesis [19][20][21]; notwithstanding, the association between *T. vaginalis* and prostate cancer remains under discussion [22][23][24].

This STI has been treated with metronidazole since 1959 [25]. Four decades later, the use of tinidazole was accepted [26][27][28], and recently the Food and Drug Administration has approved the use of secnidazole in the United States [29]. Likewise, in recent years, new recommendations on dosage in the treatment of infected women have been proposed to achieve a better rate of complete cure [28]. However, in spite of all this, almost 10% of clinical cases are resistant [30] and cross-resistance between 5-nitroimidazole drugs has been reported [31]. The absence of pharmacological alternatives to cope with treatment failure, hypersensitivity to 5-nitroimidazoles, or side effects [32][33] increases the risk of transmission and the development of chronic infection. Additionally, as trichomoniasis is highly asymptomatic and there are no diagnostic procedures to identify resistant and asymptomatic cases, the diagnosis based on syndromic management or microscopic identification of the parasite [34] hinders the correct management of the infected population. Therefore, the implementation of techniques with high sensitivity and specificity for use in routine and universal screening would reduce the incidence of this STI and, therefore, the risk associated with the acquisition of other pathologies, contributing significantly to this serious health problem.

2. Classical Diagnostic Procedures: Microscopy

Diagnosis of trichomoniasis has traditionally consisted of taking a specimen from the patient and examining it immediately under the microscope [35][36] to identify the characteristic morphology and motility of the trophozoite. The most reliable specimens for the diagnosis of female trichomoniasis include endocervical and vaginal swabs and urine [37][38][39], while in male patients are urine, urethral swabs, and semen [39][40]. Cervicovaginal and urethral specimens are obtained with cotton swabs or polyester sponges [35][36][37].

Today, wet mount microscopy is the fastest and most widely used method for diagnosing trichomoniasis in resource-limited areas [41]. This method can have a specificity of 100%; nevertheless, it must be carried out quickly enough and at a temperature that does not impair the viability of the trophozoite. It is important to note that sensitivity values may decrease depending on the time elapsed from sample collection to microscopic examination [42]. Furthermore, this subjective method achieves a sensitivity that, depending on the experience of the technician, can range between 35–80% in comparison with the culture method [43][44][45]. In addition, a delay in specimen transport reduces the motility of trichomonads, which affects the procedure's sensitivity [46][47]. This method is the most efficient diagnostic test, but its reliability and sensitivity are not optimal [44][45]. This low potential sensitivity contributes to the underdiagnosis of the disease.

Ideally, the saline wet mount preparations should be examined from the swab collected by the clinician, as well as inoculation of the samples immediately after collection in an appropriate culture medium [46][48]. Also, if samples are not immediately observed, they can be maintained in a suitable transport medium to avoid dehydration and redox potential changes. Stuart's transport culture media and its modifications are the most recommended [49][50][51]. The average survival time in these transport culture media is approximately 24 h [52]. Inadequate transport or storage conditions may reduce the parasites viability and influence the wet mount technique sensitivity [42]. In this context, permanent staining techniques were developed as a complement to the direct examination of wet mount preparations [36].

2.1. Wet Smears

Microscopic observation of vaginal exudate diluted in saline is the routine procedure for the diagnosis of female trichomoniasis; however, epithelial cells and polymorphonuclear leukocytes in the samples may interfere with the parasite's flagellar motility. The sensitivity of this technique is highly variable due to multiple causes, such as the type of sample, the number of viable organisms, and the delay between the obtention and the microscopic diagnosis, among others [43][44][45][53][54].

Regarding male samples, Feinberg and Whittington observed a greater sensitivity when direct microscopy was used with urethral material than culture methods [53]; however, there are discrepancies in other reports [55].

2.2. Staining Techniques

Different stains have been developed to increase the sensitivity of direct examination. Stained smears can be preserved without loss of diagnostic reliability due to adequate fixation and be observed later [36]. The most frequently used stains include Papanicolaou, Giemsa, and acridine orange [56][57][58][59][60][61][62]. Furthermore, less-well-known stains have also been tested, including Leishman, periodic acid–Schiff, and Fontana–Masson stains [60][61][62]. The Giemsa stain is perhaps the most accessible in the laboratory and has been used for more than 100 years in trichomoniasis diagnosis [63]. In these preparations, the nucleus of trichomonads stains purplish red and the cytoplasm is light red, pink, or bright blue, depending on pH, with a darker staining nucleus that may be oval or spindle-shaped. Sometimes, axostyle and flagella can be observed [64]. Generally, microscopic examination of Giemsa-stained smears is more effective in detecting infections than wet smear microscopy [65][66][67] and may have a sensitivity near that of culture [60][65][68].

Other stains such as safranin, methylene blue, and malachite green, which do not stain trophozoites, can act as counterstains [69][70][71]. Fluorescein can also be used to observe wet mount slides under an ultraviolet light microscope [72]. Thus, acridine orange for fluorescence-based detection of *T. vaginalis* has also been suggested by other authors as it exhibits a greater sensitivity than Giemsa staining but requires UV fluorescent light microscopy [38][73]. However, these staining methods have not been convincingly demonstrated to improve the detection rate of trichomonads in secretions and are not recommended for routine clinical diagnosis.

In Papanicolaou smears of cervicovaginal material, *T. vaginalis* exhibits an ovoid structure and an approximate size of 10–30 µm with a greenish grey cytoplasm which contains very small eosinophilic granules, and the eccentric nucleus stains blue. The sensitivity and specificity of this stain vary depending on the experience of the microscopist [42][70][74] as shown in **Table 1**.

The traditional diagnostic methodology is easy to perform; however, these techniques have the disadvantage of not being very sensitive and require careful observation by expert microscopists.

2.3. Culture

Liquid or broth culture of a clinical specimen (cervicovaginal, urethral, or urinary sediment) for microscopic observation has been considered the gold standard technique for the diagnosis of trichomoniasis, due to its sensitivity, simplicity, and the relatively low inoculum requirement (300 trichomonads/mL) [75]. Several media have been described for the *T. vaginalis* culture: Kupferberg, Kupferberg STS, Hirsch, Trichosel, Modified Diamond, Lash serum, or the most recent, called InPouch® TV [76][77][78]. However, the most common are Diamond (TYM), modified Diamond, or Roiron® [42].

Diamond's medium requires refrigeration at 4 °C for storage but should be at room temperature before specimen inoculation. Samples should be inoculated immediately into the culture medium, at least 1 h after collection, and incubated at 37 °C in anaerobic conditions (5% CO_2). This should be followed by daily examination for 3–7 days until viable trichomonads are observed [42][75]. Longer incubation times are often required in male specimens to allow the growth of a detectable number of organisms [6].

Thus, this methodology is simple and inexpensive, but requires the direct microscopic examination during a long period [40] in which infected patients may continue to transmit the infection [79]. Moreover, there are inherent limitations to culture diagnosis, e.g., culture contamination with vaginal microbiota (bacteria or yeasts) can be very frequent [80][81]. Nevertheless, sensitivity rates can rise to nearly 95% depending on the sample and the medium used (**Table 1**).

To enhance the acceptance of culture diagnosis, a good procedure is the so-called delayed inoculation, a method that combines both techniques: first, the fresh sample for direct examination and, if negative, its incubation in culture medium for 2–5 days [82]. Regarding this, InPouch® TV is a self-contained system which permits both immediate examination and culture in a single device of vaginal, urethral, and urine samples. The sensitivity is comparable to that obtained with wet smears and culture specimens [83][84]. The transparent oxygen-resistant plastic can be examined directly under the microscope, allowing daily examination of the specimen without removing it from its culture medium. InPouch® TV can be kept at room temperature, and even inoculated pouches can remain at room temperature for up to 48 h before incubation at 37 °C [42]. Levi and coworkers demonstrated that the InPouch® TV system was as sensitive as modified Diamond's medium for *T. vaginalis* detection [85]. Borchardt and collaborators demonstrated that this system is more sensitive than modified Diamond's medium or Trichosel medium [86]. However, InPouch® TV continues to be a procedure that requires observation of the sample for several days, not a rapid diagnostic technique [40][42].

Table 1. Relevant characteristics of the techniques used in the direct diagnosis of *T. vaginalis*.

Type of Diagnosis	Test	Sensitivity (Se) Specificity (Sp)	Advantages	Disadvantages	Ref.
Microscopy	Wet smears	Se: 35–85% Sp: 100%	Fast, simple, and inexpensive.	Sensitivity depends on the skills of the microscopist. Not applicable to male specimens.	[43] [44] [45]

Type of Diagnosis	Test	Sensitivity (Se) Specificity (Sp)	Advantages	Disadvantages	Ref.
					[53] [54]
Staining	Giemsa	Se: 80% Sp: 99.4%	Fast, simple, and inexpensive. Improved sensitivity vs. wet smears Stain used in Pap smears. Fast, simple, and inexpensive.	Staining specialists required to improve sensitivity.	[60] [65] [68]
	Acridine orange	Se: 100% Sp: 100%			[36]
	Papanicolaou	Se: 60–95% Sp: 98–100%			[42]
Culture	Diamond medium	Se: 56–95.8% Sp: 100%	Improved sensitivity vs. wet smears. Less handling, simple, easy to transport	Requires equipment and laboratory specialist. Risk of pathogen contamination and false negatives. Long incubation period.	[42] [83] [84]
	InPouch® [84]	Se: 92% Sp: 98%			[85] [86]

llow early based on in many

References

1. World Health Organization. Sexually Transmitted Infections (STIs). Available online: https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis) (accessed on 19 June 2023).
2. World Health Organization. Global Health Sector Strategies on, Respectively, HIV, Viral Hepatitis and Sexually Transmitted Infections for the Period 2022–2030. Available online: https://www.who.int/publications/i/item/9789240053779 (accessed on 27 September 2023).
3. Van Gerwen, O.T.; Muzny, C.A.; Marrazzo, J.M. Sexually transmitted infections and female reproductive health. Nat. Microbiol. 2022, 7, 1116–1126.
4. Rowley, J.; Vander Hoorn, S.; Korenromp, E.; Low, N.; Unemo, M.; Abu-Raddad, L.J.; Chico, R.M.; Smolak, A.; Newman, L.; Gottlieb, S.; et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: Global prevalence and incidence estimates, 2016. Bull. World Health Organ. 2019, 97, 548–562.
5. Seña, A.C.; Miller, W.C.; Hobbs, M.M.; Schwebke, J.R.; Leone, P.A.; Swygard, H.; Atashili, J.; Cohen, M.S. Trichomonas vaginalis infection in male sexual partners: Implications for diagnosis, treatment, and prevention. Clin. Infect. Dis. 2007, 44, 13–22.

6. Meites, E. Trichomoniasis: The “Neglected” Sexually Transmitted Disease. Infect. Dis. Clin. N. Am. 2013, 27, 755–764.
7. Hoots, B.E.; Peterman, T.A.; Torrone, E.A.; Weinstock, H.; Meites, E.; Bolan, G.A. A Trich-y question: Should Trichomonas vaginalis infection be reportable? Sex. Transm. Dis. 2013, 40, 113–116.
8. Centers for Disease Control and Prevention. Parasites-Parasitic Infections in the United States. Available online: https://www.cdc.gov/parasites/npi/index.html (accessed on 20 July 2023).
9. Mielczarek, E.; Blaszkowska, J. Trichomonas vaginalis: Pathogenicity and potential role in human reproductive failure. Infection 2016, 44, 447–458.
10. Lewis, D. Trichomoniasis. Medicine 2014, 43, 369–371.
11. Muzny, C.A.; Schwebke, J.R. The clinical spectrum of Trichomonas vaginalis infection and challenges to management. Sex. Transm. Infect. 2013, 89, 423–425.
12. Poole, D.N.; McClelland, R.S. Global epidemiology of Trichomonas vaginalis. Sex. Transm. Infect. 2013, 89, 418–422.
13. Van Der Pol, B. Trichomonas vaginalis infection: The most prevalent nonviral sexually transmitted infection receives the least public health attention. Clin. Infect. Dis. 2007, 44, 23–25.
14. Masha, S.C.; Cools, P.; Sanders, E.J.; Vaneechoutte, M.; Crucitti, T. Trichomonas vaginalis and HIV infection acquisition: A systematic review and meta-analysis. Sex. Transm. Infect. 2019, 95, 36–42.
15. Kissinger, P.; Adamski, A. Trichomoniasis and HIV interactions: A review. Sex. Transm. Infect. 2013, 89, 426–433.
16. Allsworth, J.E.; Ratner, J.A.; Peipert, J.F. Trichomoniasis and other sexually transmitted infections: Results from the 2001–2004 national health and nutrition examination surveys. Sex. Transm. Dis. 2009, 36, 738–744.
17. Noël, J.C.; Fayt, I.; Romero Munoz, M.R.; Simon, P.; Engohan-Aloghe, C. High prevalence of high-risk human papillomavirus infection among women with Trichomonas vaginalis infection on monolayer cytology. Arch. Gynecol. Obstet. 2010, 282, 503–505.
18. Cotch, M.F.; Pastorek, J.G.; Nugent, R.P.; Hillier, S.L.; Gibbs, R.S.; Martin, D.H.; Eschenbach, D.A.; Edelman, R.; Carey, C.; Regan, J.A.; et al. Trichomonas vaginalis associated with low birth weight and preterm delivery. The vaginal infections and prematurity study group. Sex. Transm. Dis. 1997, 24, 353–360.
19. Viikki, M.; Pukkala, E.; Nieminen, P.; Hakama, M. Gynecological infections as risk determinants of subsequent cervical neoplasia. Acta Oncol. 2000, 39, 71–75.

20. Hammar, B.; Teutsch, B.; Hoffmann, E.; Hegyi, P.; Váradi, A.; Nyirády, P.; Hunka, Z.; Ács, N.; Lintner, B.; Harmánné, R.J.; et al. Trichomonas vaginalis infection is associated with increased risk of cervical carcinogenesis: A systematic review and meta-analysis of 470 000 patients. Int. J. Gynecol. Obstet. 2023, 163, 31–43.
21. Fazlollahpour-Naghibi, A.; Bagheri, K.; Almukhtar, M.; Taha, S.R.; Zadeh, M.S.; Moghadam, K.B.; Tadi, M.J.; Rouholamin, S.; Razavi, M.; Sepidarkish, M.; et al. Trichomonas vaginalis infection and risk of cervical neoplasia: A systematic review and meta-analysis. PLoS ONE 2023, 18, e0288443.
22. Hann, I.-H.; Kim, J.-H.; Ryu, J.-S. Inflammatory response to Trichomonas vaginalis in the pathogenesis of prostatitis and benign prostatic hyperplasia. Parasit. Host. Dis. 2023, 61, 2–14.
23. Marous, M.; Huang, W.Y.; Rabkin, C.S.; Hayes, R.B.; Alderete, J.F.; Rosner, B.; Grubb, R.L.; Winter, A.C.; Sutcliffe, S. Trichomonas vaginalis infection and risk of prostate cancer: Associations by disease aggressiveness and race/ethnicity in the PLCO Trial. Cancer Causes Control 2017, 28, 889–898.
24. Nagata, M.; Tome, A.; White, K.; Wilkens, L.R.; Park, S.-Y.; Marchand, L.L.; Haiman, C.; Hernandez, B.Y. No association of Trichomonas vaginalis seropositivity with advanced prostate cancer risk in the multiethnic cohort: A nested case-control study. Cancers 2023, 15, 5194.
25. Rodin, P.; King, A.J.; Nicol, C.S.; Barrow, J. Flagyl in the treatment of trichomoniasis. Brit. J. Vener. Dis. 1960, 36, 147–151.
26. Fung, H.B.; Doan, T.-L. Tinidazole: A nitroimidazole antiprotozoal agent. Clin. Therapeut. 2005, 27, 1859–1884.
27. Sherrard, J.; Wilson, J.; Donders, G.; Mendling, W.; Jensen, J.S. 2018 European (IUSTI/WHO) International Union against sexually transmitted infections (IUSTI) World Health Organization (WHO) guideline on the management of vaginal discharge. Int. J. STD AIDS 2018, 29, 1258–1272.
28. Kissinger, P.J.; Gaydos, C.A.; Seña, A.C.; McClelland, R.C.; Soper, D.; Secor, W.E.; Legendre, D.; Workowski, K.A.; Muzny, C.A. Diagnosis and management of Trichomonas vaginalis: Summary of evidence reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections treatment guidelines. Clin. Infect. Dis. 2022, 74, S152–S161.
29. Muzny, C.A.; Van Gerwen, O.T. Secnidazole for Trichomoniasis in women and men. Sex. Med. Rev. 2022, 10, 255–262.
30. Kissinger, P. Trichomonas vaginalis: A review of epidemiologic, clinical and treatment issues. BMC Infect. Dis. 2015, 15, 307.
31. Dunne, R.L.; Dunn, L.A.; Upcroft, P.; O'Donoghue, P.J.; Upcroft, J.A. Drug resistance in the sexually transmitted protozoan Trichomonas vaginalis. Cell Res. 2003, 13, 239–249.

32. Helms, D.J.; Mosure, D.J.; Secor, E.; Workowski, K.A. Management of Trichomonas vaginalis in women with suspected metronidazole sensitivity. Am. J. Obstet. Gynecol. 2008, 198, 370.e1–370.e7.
33. Forna, F.; Gülmezoglu, A.M. Interventions for treating trichomoniasis in women. Cochrane Database Syst. Rev. 2003, 2, CD000218.
34. Gaydos, C.A.; Klausner, J.D.; Pai, N.P.; Kelly, H.; Coltart, C.; Peeling, R.W. Rapid and point-of-care tests for the diagnosis of Trichomonas vaginalis in women and men. Sex. Transm. Infect. 2017, 93, S31–S35.
35. Krieger, J.N. Trichomoniasis in men: Old issues and new data. Sex. Transm. Dis. 1995, 22, 83–96.
36. Khatoon, R.; Jahan, N.; Khan, H.M.; Rabbani, T.; Ahmad, S. Evaluation of different staining techniques in the diagnosis of Trichomonas vaginalis infection in females of reproductive age group. J. Clin. Diagn. Res. 2014, 8, 5–8.
37. Robertson, D.H.H.; Lumsden, W.H.R.; Fraser, K.F.; Hosie, D.D.; Moore, D.M. Simultaneous isolation of Trichomonas vaginalis and collection of vaginal exudates. Br. J. Vener. Dis. 1969, 45, 42–43.
38. Oates, J.K.; Selwyn, S.; Breach, M.R. Polyester sponge swabs to facilitate examination for genital infection in women. Br. J. Vener. Dis. 1971, 47, 289–292.
39. Galán-Montemayor, J.C.; Lepe-Jiménez, J.A.; Otero-Guerra, L.; Serra-Pladevall, J.; Vázquez-Valdés, F. Diagnóstico microbiológico de las infecciones de transmisión sexual y otras infecciones genitales. In Procedimientos en Microbiología Clínica, 24th ed.; Cercenado-Mansilla, E., Cantón-Moreno, R., Eds.; Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC): Madrid, Spain, 2018; pp. 1–94. ISBN 978-84-09-07783-0.
40. Alcamo, I.E. Fundamentals of Microbiology, 6th ed.; Jones and Bartlett Publishers: Boston, MA, USA, 2001; pp. 486–487. ISBN 978-0-7637-0067-6.
41. Madhivanan, P.; Li, T.; Trammell, S.; Desai, C.; Srinivas, V.; Arun, A.; Klausner, J.D.; Krupp, K. Performance of the OSOM Trichomonas Rapid Test for diagnosis of Trichomonas vaginalis infection among women in Mysore. Indian Sex. Health 2013, 10, 320–324.
42. Hobbs, M.M.; Seña, A.C. Modern diagnosis of Trichomonas vaginalis infection. Sex. Transm. Infect. 2013, 89, 434–438.
43. Beverly, A.L.; Vengtarik, L.M.; Cotton, B.; Schwebke, J.R. Viability of Trichomonas vaginalis in transport medium. J. Clin. Microbiol. 1999, 37, 3749–3750.
44. Martin, R.D.; Kaufman, R.H.; Burns, M. Trichomonas vaginalis: A statistical evaluation of diagnostic methods. Am. J. Obstet. Gynecol. 1963, 87, 1024–1027.

45. McCann, J.S. Comparison of direct microscopy and culture in the diagnosis of trichomoniasis. Br. J. Vener. Dis. 1974, 50, 450–452.
46. Clark, D.H.; Solomons, E. An evaluation of routine culture examination for Trichomonas vaginalis and Candida. Am. J. Obstet. Gynecol. 1959, 78, 1314–1319.
47. Kingston, M.A.; Carlin, D.; Bansal, E.M. Shelf life of Trichomonas vaginalis. Int. J. STD AIDS 2003, 14, 28–29.
48. McMillan, A. Laboratory diagnostic methods and cryopreservation of trichomonads. In Trichomonads Parasitic in Humans; Honigberg, B.M., Ed.; Springer: New York, NY, USA, 1990; pp. 297–310.
49. Stuart, R.D. Transport problems in public health bacteriology. Can. J. Public Health 1956, 47, 114–122.
50. Bolumburu, C.; Zamora, C.; Muñoz-Algarra, M.; Portero-Azorín, F.; Escario, J.A.; Ibáñez-Escribano, A. Trichomoniasis in a tertiary hospital of Madrid, Spain (2013–2017): Prevalence and pregnancy rate, coinfections, metronidazole resistance, and endosymbiosis. Parasitol. Res. 2020, 119, 1915–1923.
51. Amies, C.R. A modified formula for the preparation of Stuart’s transport medium. Can. J. Public Health 1967, 58, 296–300.
52. Nielsen, R. Trichomonas vaginalis. Survival in solid Stuart’s medium. Br. J. Vener. Dis. 1969, 45, 328–331.
53. Feinberg, J.G.; Whittington, M.J. A culture medium for Trichomonas vaginalis Donné and species of Candida. J. Clin. Pathol. 1957, 10, 327–329.
54. Eriksson, G.; Wanger, L. Frequency of N. gonorrhoeae, T. vaginalis and C. albicans in female venereological patients. Br. J. Vener. Dis. 1975, 51, 192–197.
55. Hoffman, B.; Kilczewski, W.; Malyszko, E. Studies in trichomoniasis in males. Br. J. Vener. Dis. 1961, 37, 172–175.
56. Hughes, H.E.; Gordon, A.M.; Barr, G.T.D. A clinical laboratory study of trichomoniasis of the female genital tract. Br. J. Obstet. Ginecol. 1966, 73, 821–827.
57. Freeman, F. A modified staining technique for Trichomonas vaginalis. S. Afr. Med. J. 1958, 32, 1235.
58. Lowe, G.H. The laboratory diagnosis of trichomoniasis. In Laboratory Diagnosis of Venereal Disease; Public Health Laboratory Service Monograph Series No. 1; H. M. Stationery Office: London, UK, 1972; pp. 40–42.

59. Fripp, P.J.; Mason, P.R.; Super, H. A method for the diagnosis of Trichomonas vaginalis using acridine orange. J. Parasitol. 1975, 61, 966–967.
60. Levett, P.N. A comparison of five methods for the detection of Trichomonas vaginalis in clinical specimens. Med. Lab. Sci. 1980, 37, 85–88.
61. Rodriguez-Martinez, H.A.; De la Luz Rosales, M.; Gallaso de Bello, L.; Ruiz-Moreno, J.A. Adequate staining of Trichomonas vaginalis by McManus' periodic acid-Schiff stain. Am. J. Clin. Pathol. 1973, 59, 741–746.
62. Nagesha, C.N.; Ananthkrishna, N.C.; Sulochana, P. Clinical and laboratory studies on vaginal trichomoniasis. Am. J. Obstet. Gynecol. 1970, 106, 933–935.
63. Tanabe, M. Morphological Studies on Trichomonas. J. Parasitol. 1926, 12, 120–130.
64. Sorel, C. Trois techniques de recherche du Trichomonas vaginalis: Leurs valeurs comparées. Presse Med. 1954, 62, 602–604.
65. Lowe, G.H. A comparison of current laboratory methods with a new semisolid culture medium for the detection of Trichomonas vaginalis. J. Clin. Pathol. 1965, 18, 432–434.
66. Rogers, S.; Goldsmid, J.M. A study of the possible value of acridine orange-O stain in the diagnosis of Trichomonas vaginalis infection. Cent. Afr. J. Med. 1977, 23, 56–58.
67. Buharowski, K.; Wolanska, M. Usefulness of staining specimens with acridine orange in the diagnosis of Trichomonas vaginalis. Wiad. Parazytol. 1984, 30, 499–501.
68. Mason, P.R.; Super, H.; Fripp, P.J. Comparison of four techniques for the routine diagnosis of Trichomonas vaginalis infection. J. Clin. Pathol. 1976, 29, 154–157.
69. Miller, J.R. Contrast stain for the rapid identification of Trichomonas vaginalis. Am. J. Clin. Pathol. 1937, 7, 40.
70. Eddie, D.A.S. The laboratory diagnosis of vaginal infections caused by Trichomonas and Candida (Monilia) species. J. Med. Microbiol. 1968, 1, 153–159.
71. Perju, A.; Strîmbeanu, I. Valeur de la spermoculture dans ie diagnostic de la trichomonase urogenitale chez l'homme. Wiad. Parazytol. 1966, 12, 475–480.
72. Coutts, W.E.; Silva-Inzunza, E. Vital staining of Trichomonas vaginalis with fluorescein. Br. J. Vener. Dis. 1954, 30, 43–46.
73. Bickley, L.S.; Krisher, K.K.; Punsalang, A.; Trupei, M.A.; Reichman, R.C.; Menegus, M.A. Comparison of direct fluorescent antibody, acridine orange, wet mount, and culture for detection of Trichomonas vaginalis in women attending a public sexually transmitted diseases clinic. Sex Transm Dis. 1989, 16, 127–131.

74. Thin, R.N.T.; Atia, W.; Parker, J.D.J.; Nicol, C.S.; Canti, G. Value of Papanicolaou-stained smears in the diagnosis of trichomoniasis, candidiasis and cervical herpes simplex virus infection in women. Br. J. Vener. Dis. 1975, 51, 116–118.
75. Garber, G.E.; Sibau, L.; Ma, R.; Proctor, E.M.; Shaw, C.E.; Bowie, W.R. Cell culture compared with broth for detection of Trichomonas vaginalis. J. Clin. Microbiol. 1987, 25, 1275–1279.
76. Thomason, J.L.; Gelbart, S.M.; Sobun, J.F.; Schulien, M.B.; Hamilton, P.R. Comparison of four methods to detect Trichomonas vaginalis. J. Clin. Microbiol. 1988, 26, 1869–1870.
77. Schmid, G.P.; Matheny, L.C.; Zaidi, A.A.; Kraus, S.J. Evaluation of six media for the growth of Trichomonas vaginalis from vaginal secretions. J. Clin. Microbiol. 1986, 27, 1230–1233.
78. Patil, M.J.; Nagamoti, J.M.; Metgud, S.C. Diagnosis of Trichomonas vaginalis from vaginal specimens by wet mount microscopy, InPouch TV culture system, and PCR. J. Glob. Infect. Dis. 2012, 4, 22–25.
79. Moldwin, R.M. Sexually transmitted protozoal infections. Trichomonas vaginalis, Entamoeba histolytica, and Giardia lamblia. Urol. Clin. N. Am. 1992, 19, 93–101.
80. Fouts, A.C.; Kraus, S.J. Trichomonas vaginalis: Reevaluation of its clinical presentation and laboratory diagnosis. J. Infect. Dis. 1980, 141, 137–143.
81. Van Gerwen, O.T.; Muzny, C.A. Recent advances in the epidemiology, diagnosis, and management of Trichomonas vaginalis infection. F1000Res 2019, 8, 1666.
82. Schwebke, J.R.; Burgess, D. Trichomoniasis. Clin. Microbiol. Rev. 2004, 17, 794–803.
83. Sood, S.; Mohanty, S.; Kapil, A.; Tolosa, J.; Mittal, S. InPouch TV culture for detection of Trichomonas vaginalis. Indian J. Med. Res. 2007, 125, 567–571.
84. Méndez-Izquierdo, J.E.; Fonseca-Berzal, C.R.; Ibáñez-Escribano, A. Trichomonas vaginalis: Diagnosis of a forgotten sexually transmitted infection. An. Real Acad. Farm. 2023, 89, 135–148.
85. Levi, M.H.; Torres, J.; Pina, C.; Klein, R.S. Comparison of the InPouch system and Diamond´s modified medium for detection of Trichomonas vaginalis. J. Clin. Microbiol. 1996, 35, 3308–3310.
86. Borchardt, K.A.; Zhang, M.; Shing, H.; Flink, K. A comparison of the sensitivity of the InPouch TV, Diamond’s and Trichosel media for the detection of Trichomonas vaginalis. Genitourin. Med. 1997, 73, 297–298.