Original Research Article

Comparison of three diagnostic methods for *Trichomonas vaginalis* detection in a low-resource setting

ABSTRACT

Aims: To comparatively evaluate the efficiency of three simple trichomoniasis diagnostic techniques in a low-resource setting at the Baixada Fluminense region, Province of Rio de Janeiro, Brazil.
Place and Duration of Study: The sample was obtained from the medical records of women attending the Iguaçu University Clinic's Gynaecology Outpatient Clinic from January to December 2020. The sample consisted of medical records of 135 women aged 17 to 78 years.
Methodology: With the aid of a sterile and disposable bi-valve speculum, vaginal secretion was collected with a swab from the vaginal sac. The secretion was used for smears in two slides for fresh examination and Papanicolaou staining and then seeded in a tube with 6 ml of Diamond's culture medium.
Results: The results showed that among the 135 women with clinical signs of vulvovaginitis who attended at the Gynecology Outpatient Clinic of the Iguaçu University, 65 (48.15%) were infected by *Trichomonas vaginalis*. The positivity rates displayed significant differences according to the detection methods used. Wet mount examination and Papanicolaou exams were less efficient, detecting only 7.7% of total infections confirmed by the culture in Diamond's medium.
Conclusion: The analysis of the results allowed an update in the detection protocol of *Trichomonas vaginalis* infections in the Gynecology Outpatient Clinic of the Iguaçu University. Papanicolaou test was eliminated from the protocol since it requires more resources than wet mount examination. Wet mount examination was maintained because it detects part of the *Trichomonas vaginalis* infections at the time of medical attending, following the core principle of the syndromic approach indicated by WHO, but with greater efficacy than the diagnostic determination based on signs and symptoms. Culture by Diamond's medium proved to be one of the best options for low-resource settings when compared to other detection methods of *T. vaginalis* analyzed in the current scientific literature, with an acceptable detection rate, only lower than sophisticated and high-cost methods.

Keywords: Trichomonas vaginalis, Detection Methods, Wet Mount Examination, Papanicolaou, Diamond Culture Media, Sexually Transmitted Infection, Laboratorial Methods Comparison, Low-Resources Settings

1. INTRODUCTION

Trichomonas vaginalis is a flagellated parasitic protozoan first described in 1836 by the French physician Alfred François Donné, when he observed a sample of vaginal fluid under a microscope. He named this new microorganism *Trichomonas*, in allusion to the flagella that cover the cell like hair. *T. vaginalis* was considered a commensal until the 1950s, when it was recognised as a pathogen causing a sexually transmitted disease [1].
Trichomonas vaginalis is a eukaryotic parasitic protozoan, generally oval or fusiform in shape. It presents in the form of a trophozoite. However, the parasite cell can mutate to an amoeboid or pseudocysts appearance when attaching to surfaces of the host epithelium [1,2,3]. It measures approximately 10 µm in length by 7 µm in width [1,4]. This protozoan

has four unequal flagella that originate from the same point, an undulating membrane which brings mobility, and a rigid structure that projects through the centre of the cell to the posterior end called the axostyle [1,2,3,5]. The entire cell surface is capable of performing phagocytosis of bacteria, leukocytes and other particles. This protozoan can live and multiply under conditions of aerobiosis and anaerobiosis [4,5].

Trichomoniasis is an infection of the urogenital tract caused by *Trichomonas vaginalis*, causing vulvovaginitis and cervicitis in women [3,6]. In men, it can cause urethritis, epididymitis and prostatitis [6,7]. Usually, the clinical picture begins with urethral itching in men or vaginal itching in women, abdominal discomfort and dysuria. The female gender is the most affected by symptomatic infections, and vaginal discharge, greenish-yellow, sometimes frothy and with a foul-smelling odour, is the most frequent manifestation. Other clinical manifestations include vulvar erythema, excessive discharge, inflammation and erosion of the vaginal wall and cervix, sometimes with a "strawberry" appearance on colposcopic examination. More extensive involvement in males is uncommon, but possible. It is estimated that between 70% and 85% of individuals infected with *Trichomonas vaginalis* are asymptomatic [5,6]. After infection, individuals may show symptoms between 5 and 28 days [5]. *Trichomonas vaginalis* infection is associated with an increased risk of cervical cancer, premature birth and increased risk for HIV infection [2,3,5].

Trichomonas vaginalis has a specific affinity for the urogenital tract and has receptors and proteins in its membrane for adhesion to the vaginal and urethral epithelium, secreting adhesins, proteases and vesicles with toxins that facilitate intercellular penetration and initiate the inflammatory process, which results in the secretion of unpleasant-smelling fluid [3,5]. The intensity and severity of infection depend on the number of parasites and the toxicity of the *T. vaginalis* strain, the reduction of the *Lactobacillus* flora and consequently the elevation of the normal pH of the area. This protozoan grows at pH between 5.0 and 7.5 and temperatures ranging from 20 to 40 °C [2,3].

Trichomoniasis is the most prevalent non-viral sexually transmitted disease in the world [3,5,8,9], affecting approximately 400 million people and with new infections reported at about 156 million annually [3,9]. The prevalence of trichomoniasis is higher among women in countries with lower relative development [8,9,10], and populations living in areas of poor health resources may have high incidence rates [11]. Currently, it is recommended that in medical care in regions with low resource conditions, investigation methods for reliable and inexpensive diagnostics and screening tests should be applied [12,13,14,15]. This research aims to comparatively evaluate the efficiency of three simple techniques for trichomoniasis diagnosis in a low-resource setting in the Baixada Fluminense region, Province of Rio de Janeiro, Brazil.

2. MATERIAL AND METHODS

2.1 SAMPLED POPULATION AND RECRUITMENT

A retrospective research was conducted from samples obtained from the set of medical records of women who voluntarily sought care at the Gynaecology Outpatient Clinic of the Iguaçu University Clinic in the period from January to December 2019. The sample consisted of medical records of 135 women aged between 17 and 78 years. The entire research procedure was guided by anonymity and non-discrimination, fully following the precepts of national and international ethical standards, and completely in accordance with the standards determined by the Helsinki Declaration of 1964.

2.2 INCLUSION CRITERIA

Medical records of women seen at the Gynaecology Outpatient Clinic of the Iguaçu University Clinic who presented clinical signs of vulvovaginitis were included in the research. The records of patients who were under treatment with nitroimidazole or some type of gynaecological procedure were excluded from the research.

2.3 SAMPLE COLLECTION, MATERIALS AND METHODS

The clinic has its own protocol for the diagnosis of trichomoniasis, which consists of the microscopic examination of fresh preparation, Papanicolaou staining and culture of the material in Diamond medium.

The standard procedure for obtaining the discharge sample is to use a sterile, disposable bivalve speculum and to collect vaginal secretion from the vaginal sac with a swab. The collected secretion is used for smears on two light microscopy slides and then seeded in a tube with 6 ml of Diamond's culture medium.

For the fresh preparation, immediately after sample collection, the secretion is spread on one of the slides, and 0.85% saline solution is added and covered with a coverslip. The slides are examined under an optical microscope with a 40x magnification objective, and a direct examination for *Trichomonas vaginalis is* performed.

For Papanicolaou's exam, the second slide is taken to the laboratory for staining. The slide is analysed under an optical microscope with an objective lens magnification of 100x, and a visual examination for *Trichomonas vaginalis* is performed.

For the culture of *Trichomonas vaginalis*, Diamond's culture medium marketed by the company Dalynn Biologicals was used. The culture medium is prepared and heated at 35°C in an incubator before inoculation. Seeding is performed

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immediately after collecting the material with a swab in circular movements over the medium. The tube is closed and placed in the incubator at 37°C. The material is examined daily for five days. Each day, a drop from the lower portion of the tube is examined under a microscope for possible observation of motile trophozoites of *Trichomonas vaginalis*.

2.4 STATISTICAL ANALYSIS

The results of the three methods used for the diagnosis of vulvovaginal trichomoniasis were compared to each other for the proportion of positivity using the "Z" test, with a significance level of 5% (u = 0.05).

3. RESULTS AND DISCUSSION

The results showed that among 135 women with clinical signs of vulvovaginitis who sought care at the Gynecology Outpatient Clinic of Iguaçu University, a total of 65 patients (48.15%) were infected by *Trichomonas vaginalis*. The positivity presented significant differences according to the detection methods used.

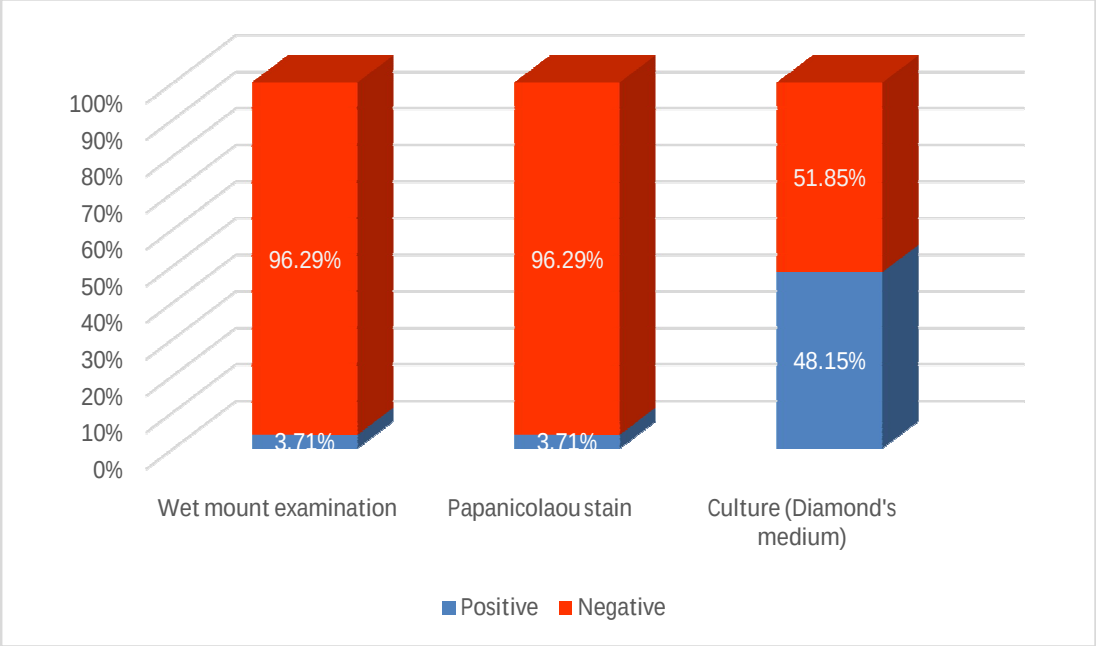

Fig. 1. Comparison of the results obtained for the detection of *Trichomonas vaginalis* by direct examination, Papanicolaou staining and culture on Diamond medium of samples from 135 women seen at the Gynaecology Outpatient Clinic of the Iguaçu University, Rio de Janeiro State, Brazil

The analysis shows that the fresh Papanicolaou and Pap tests obtained 44.44% of false-negative results, 3.71% of true-positives; 51.85% of true-negatives and 0% of false-positives. These results demonstrate the low sensitivity of the two techniques, but high specificity. The positive cases for vaginal trichomoniasis were coincident in the fresh examination, Papanicolaou staining and culture in Diamond's medium, confirming the specificity of the methods. Based on the indexation of positive results by those confirmed by Diamond's culture, the low sensitivity of the two microscopy techniques in comparison with culture in Diamond's medium becomes even more evident (Fig. 2).

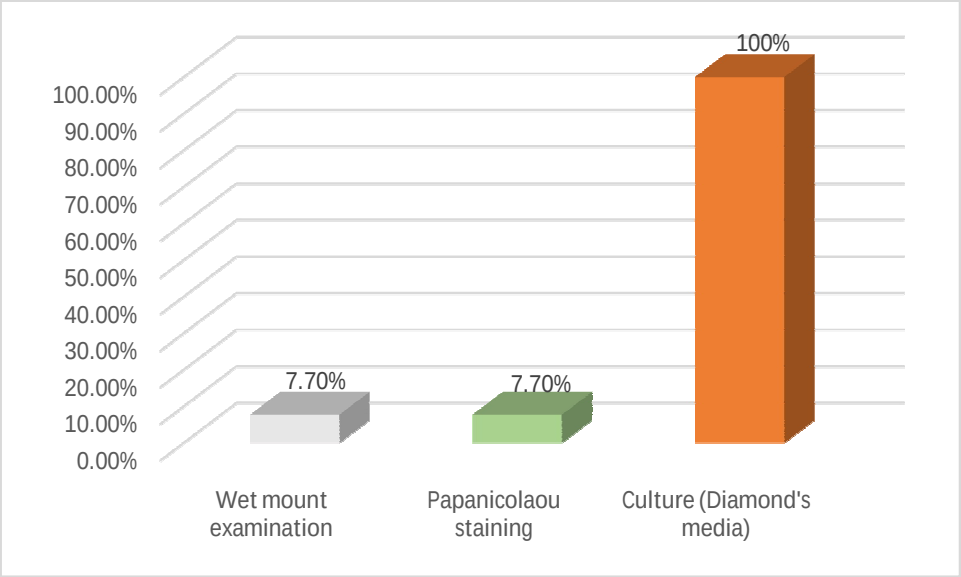

Fig. 2. Comparison of *Trichomonas vaginalis* detection efficiency indexed by the total confirmed by the culture method in Diamond's medium (gold-standard) of the 65 positive cases of trichomoniasis among women seen at the Gynecology Outpatient Clinic of the Iguaçu University, Rio de Janeiro State, Brazil

Regarding the clinical picture, 100% of patients with trichomoniasis presented vaginal discharge; in 53.84% the discharge was light-coloured (yellow or white) and 7.69% was greenish with bubbles. Among the patients, other relevant clinical signs were: hematuria (7.69%) and hyperemia of the vaginal mucosa (30.76%).
There is scarce scientific literature on comparing the efficiency of *T. vaginalis* detection methods, and the existing research is not uniform regarding the methods investigated. An overview of available articles is listed in Table 1.

Table 1. Efficiency of detection methods of *Trichomonas vaginalis* calculated through Higher Real Positivity Method (HRPM) comparison

Authors	Wet mount examination	Papanicolaou staining	Culture media	Other methods
Martin et al. [16]	65%	-	Feinberg-Whittington culture medium - 100%.	Orange acridine staining - 57 Ortho diluent - 69
Mason et al. [17]*	-	70%	Feinberg-Whittington culture medium - 97%.	Giemsa staining - 96%. Orange acridin staining - 93%.
Chintana et al. [18]	93,9%	40,6%	Cystein-Pepton-Maltose culture medium - 100%.	-
Caliendo et al. [19]	-	-	InPouch culture system-- 66.6%.	PCR - 100%
Radonjic et al. [20]*	51,8%	-	Diamond culture medium - 77.8%.	Giemsa staining - 40.7%. Orange acridine staining - 59.2%. PCR -81.5
Nye et al. [21]	69,6%	-	InPouch culture system - 93.8%.	PCR - 98.6%. APTIMA - 100%
Rezaeian et al. [22]	81,3%	-	Dorsse culture medium - 100	-
Perazzi et at. [23]	45,8%		Modified thioglycolate medium - 100%.	Giemsa staining - 58.3%. SAF/methylene blue staining - 62.5%.
Al-Saeed [24]	43,5%	73,9%	Diamond culture medium - 100%.	Hematoxylin-eosin staining - 65.2%.
Mushref et al. [25]	66,7%	-	Kupferberg culture medium -	Indirect agglutination -

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			60 Trichomonas modified CPLM - 100%.	40%. ELISA - 73.3%.
Patil et al. [26]	50%	-	InPouch culture system - 66.6%.	PCR TVK3 and PCR TVK7 - 100%.
Elsherif and Youssef [27]	51,7%	-	Diamond culture medium - 100%.	-
Khatoon et al. [28]	66,2%	-	Kupferberg culture medium - 100	OSOM test - 92.6%. Orange acridine staining - 77.9%.
Nathan et al. [29]	39,1%	-	InPouch culture system - 91.3%.	OSOM Trichomonas test - 95.6 PCR - 95.6 APTIMA test - 100
Herath et al. [30]	35,3%	-	Trichomonas culture medium Oxoid 2 - 76,5%.	PCR TVK3 - 88.2% PCR TV16Sf and PCR TFR1/2 - 100%.
Nasir et al. [31]	38,5%	-	Diamond culture medium - 80.8%. InPouch culture system - 96.2%.	Giemsa staining - 65.4%. Orange acridine staining - 69,2%

**Calculated through overall sample compared results*

The World Health Organization advocates the use of the syndromic approach as a health policy to combat sexually transmitted diseases in developing countries. This is a reactive strategy, which aims at the immediate treatment of the patient in the first health care service based on signs and symptoms, without the need for laboratory tests that prove the aetiology of the disease, eliminating costs and with the goal of avoiding the spread of the disease [32, 33]. This strategy, however, has been disqualified by several researchers, and the syndromic treatment of vaginal discharge has been considered unsatisfactory, especially in trichomoniasis [34,35,36,37,38,39]. Thus, the adoption of efficient, low-cost, low-complexity diagnostic protocols that are appropriate for the lack of medical and laboratory infrastructure in developing countries is an important advance for the health strategy of the neediest populations.

Trichomoniasis is the most prevalent non-viral sexually transmitted disease in the world. Despite the high prevalence, definitive diagnosis for infection by this protozoan in low-resource settings is still a challenge. According to Rahmani et al. [40], the methods currently used for the diagnosis of trichomoniasis are wet mount examination, culture, Papanicolaou test (staining), Acridine Orange (staining), Gram (staining), Giemsa (staining), Affirm VPIII test (molecular assay), IFA (antigen reaction), OSOM Trichomonas test (immunochromatographic), ELISA (nucleic acid amplification), AmpliVue assay (isothermal helicase-dependent amplification), Solana Trichomonas assay (nucleic acid amplification), Polymerase Chain Reaction (nucleic acid amplification), APTIMA T. vaginalis assay (specific rRNA transcriptional amplification), GeneXpert T.V assay (specific rRNA transcriptional amplification), Potassium Hydroxide (KOH) "Whiff Test", vaginal pH Test, and latex agglutination test. Each of these methods has comparative advantages and disadvantages, with different degrees of specificity and sensitivity. The simplest methods are wet mount examination, culture, Papanicolaou test (staining), Acridine Orange (staining), Gram (staining), Giemsa (staining), Potassium Hydroxide (KOH) "Whiff Test", vaginal pH Test, and latex agglutination test. The Potassium Hydroxide, vaginal pH, and latex agglutination tests are highly non-specific and cannot be considered diagnostic determinants of trichomoniasis. The wet mount examination is highly specific and of low sensitivity, but of immediate performance and practically inexpensive. Microscopy is a method recognised for its low accuracy in detecting *Trichomonas vaginalis*, although it is one of the most widely used techniques worldwide. Papanicolaou's test is traditionally used for the diagnosis of alternations in the uterine cervix and combines practicality and low cost, although it is highly nonspecific for *Trichomonas vaginalis*. According to Radonjic et al. [20], the use of staining techniques is only justifiable when the use of the fresh examination method is not possible. This proposition was confirmed in our study, as the detection rate of *T. vaginalis* was the same for the fresh examination and the Pap test. The culture of *T. vaginalis* is still considered a gold standard diagnostic method, however, it is rarely performed in the laboratory routine due to the time required for results, costs and the need for an incubation oven. Molecular tests, immunochromatography and nucleic acid amplification are highly sensitive, however, they are not always available to the health service, are expensive and often require laboratory infrastructure not available in low-resource settings [29].

Culture using Diamond's medium is considered highly efficient, allowing rapid growth of *T. vaginalis* even when the inoculum contains few microorganisms [41,42], achieving growth rates higher than those observed in Kupfelberg's medium [43], used in past decades for the culture of *T. vaginalis*. The sensitivity of the InPouch method is comparable to that of Diamond's medium [44]. Culture in the InPouch method offers some advantages over culture on Diamond's medium. After inoculation of the InPouch medium, microscopic observation can be made directly through the wrapping,

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dispensing with the need to take a sample for observation on a slide. When available in low-resource environments, it offers greater practicality with efficiency comparable to culture on Diamond's medium. A review of the scientific literature allows us to state that both culture media can be considered the gold standard in low-resource conditions, surpassed only by PCR or rRNA amplification (APTIMA) tests [20,21,26,29,30,31], which are unfeasible due to the high cost for laboratory practice in places with few economic and medical infrastructure resources. The comparison of the diagnostic results in our research with those observed in the scientific literature confirms that Diamond's medium is, among the low-cost options, the most suitable diagnostic method for its high sensitivity and specificity, with efficiency rates similar to those of the InPouch culture method.

4. CONCLUSION

The analysis of the results allowed an update in the protocol for the detection of *Trichomonas vaginalis* infections in the Gynaecology Outpatient Clinic of the Iguaçu University Clinic. The fresh preparation and Papanicolaou tests showed high specificity and low sensitivity, with the same detection rate. The Papanicolaou test was eliminated from the protocol because it required more resources than the fresh examination. The wet examination was maintained because it detected part of the *Trichomonas vaginalis* infections at the time of the consultation, as recommended by the syndromic approach indicated by the WHO, but with greater efficiency than the diagnostic determination based on signs and symptoms. The culture in Diamond's medium proved to be one of the best options for environments with few resources when compared to other means of detection of *T. vaginalis* analyzed in the current scientific literature, with an acceptable detection rate, only lower than that of sophisticated, high-cost methods.

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