

Parasitological Studies on *Trichomonas Vaginalis* on Female Patients Presented with Vaginal Discharge at Aswan University Hospital

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Abstract

Background: *Trichomonas vaginalis* (*T.vaginalis*) is one of the most common parasitic non viral Sexually Transmitted Infections (STI) in the world. The World Health Organization (WHO) has estimated that 160 million trichomoniasis cases are acquired annually worldwide [1].

Aim of Study: Evaluate different direct laboratory methods used in diagnosis of vaginal trichomoniasis.

Material and Methods: The cross sectional study was carried out on 200 female patients, suffering from vaginal discharge, aged between 20-50 years old. Two vaginal swabs were taken from every patient after taking a consent. All the samples were examined by wet mount, stained by Giemsa, rapid test and Culture on modified Diamond's medium and ELIZA.

Results: This study showed that ELIZA is the gold standard among diagnostic techniques as it detected *T. vaginalis* in 15% of suspected patients followed by 13.5% by culture, then 12.5% by rapid test and lastly wet mount which detected only 5% of cases. ELIZA and Culture gave the highest sensitivity, specificity, accuracy, NPV and PPV followed by rapid test. The least sensitive was wet mount. The least specific was Giemsa stain.

Conclusion: The study showed that wet mount method is simple and cheap. Wet mount microscopy is the preferred option for prompt diagnosis of trichomoniasis. It is proved that rapid test is relatively simple to carry out, shows reasonable sensitivity and culture remains the most accurate single method for detecting the presence of *T. vaginalis* in patient samples. and Diamond's medium is a suitable culture method and is considered as the gold standard of diagnosis of trichomoniasis.

Key Words: *Trichomonas – Vaginalis rapid test vaginal discharge – ELIZA – Diamond's medium – Aswan.*

Introduction

TRICHOMONAS vaginalis (*T.vaginalis*) is one of the most common parasitic non viral Sexually

Transmitted Infections (STI) in the world. The World Health Organization (WHO) has estimated that 160 million trichomoniasis cases are acquired annually worldwide [1]. The most risky populations for trichomoniasis are teenage, black races, pregnant women and sexually active females. It is more common in females (2.7%) than males (1.4%). Studies which were done in Egypt showed a prevalence rate range from 5% to 9.3% [2].

Trichomoniasis a common worldwide sexually transmitted disease caused by the flagellated protozoan *Trichomonas vaginalis* (*T. vaginalis*). It can be considered as a re-emerging infectious disease. The prevalence of trichomoniasis estimated between 2012 and 2016 by WHO, is 110.4 million cases. *Trichomonas vaginalis* affects the urogenital tract of male and female especially at childbearing period [3].

The disease stages vary from an asymptomatic carrier state to obvious vaginitis *T. vaginalis* infection is associated with cervicitis, urethritis and serious complications as Pelvic Inflammatory Disease (PID) infertility cervical cancer and HIV transmission. In men, trichomoniasis causes urethritis, complicated with epididymitis, prostatitis and infertility. In addition, *T.vaginalis* may be associated with cancer prostate. Although, there are different methods for diagnosis of this infection, as staining techniques, immunochromatography and nucleic acid amplification the gold standard method for diagnosis of *T. vaginalis* is the culture on Diamond's medium [4].

Subjects and Methods

Study population: This is a cross-sectional study was carried out in the outpatient clinic of Aswan

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University Hospital from March 2018 to March 2019. study to evaluate different laboratory method for diagnosis of *T.vaginalis*. Two hundred female suffering from vaginal discharge were randomly selected. Inclusion criteria were females of reproductive age group (15-45 years) attending gynecological out-patient clinic complaining of vaginal discharges, pruritus, dysuria, dyspareunia and pain in lower abdomen.

Samples collection: Each patient was subjected to PV examination and non-lubricated sterile speculum examination. After testing the PH of vagina, four sterilized cotton swabs were collected by using sterile bacteriological swabs from the posterior fornix and end cervix. Samples were labeled and rapidly transported to the Medical Parasitology Laboratory for processing.

Processing and examination of the samples as follow:

1- Direct wet mount microscopic examination [5]: Within 5 minutes of sample collection, a swab containing the vaginal sample swab was examined for at least 3 minutes using a compound microscope to confirm characteristic morphology and jerky movement of the organism. The preparation must be examined as soon as possible after the collection of specimen.

2- Giemsa staining technique: The saline tube containing the secretion was centrifuged for about 10 minutes at medium speed and stained according and one drop of the vaginal swab-PBS suspension was applied on a microscopic glass slide, air dried, fixed in methanol for 1 minute, stained with 20% Giemsa for 20 minutes. The slide was rinsed by water to remove excess stain, drained in a vertical position and left to dry and examined microscopically by X100 to detect trophozoites [6].

3- Modified Diamond's cultural [7]: The third swab was immersed immediately into the culture tube containing 5.0ml Diamond's medium at 37°C incubator for 7 days. The culture was examined microscopically on days 2, 4, 6, after inoculation. A positive result was defined as the presence of motile trichomonas at any time. While, a negative result. Culture still revealing no growth i.e. absence of motile trichomonas at all reading.

4- OSOM Trichomonas Rapid test: The test was performed according to manufacturer's instructions [8]. Vaginal swab was placed in a flexible plastic test tube containing 0.5ml of sample buffer, mixed vigorously, and allowed to stand for 1min. The test dipstick was placed in each tube containing the buffer-sample mixture; the results were read

after 10min. A positive result was indicated by the presence of a blue test line along with end.

5- Enzyme linked immunoassay (ELIZA) [9]: Polystyrene microtiter plates were coated with affinity-purified rabbit anti-*T. vaginalis* antibodies, blocked by incubation with 1% bovine serum albumin in PBS, and stored at 4°C until needed. Assay of the clinical described previously samples was done according to [9].

Results

ELIZA is the most sensitive diagnostic techniques as it detected *T. vaginalis* in 15% of suspected patients followed by Culture 13.5% by, then 8% by Rapid test and lastly wet mount which detected only 6.5% of cases (Table 1).

Table (1): The prevalence of vaginal trichomoniasis among cases using different diagnostic techniques.

Procedures	T. vaginalis positive cases	
	Number (No.)	%
Wet mount	10	5
Giemsa stain	15	7.5
Culture	27	13.5
Rapid test	25	12.5
Eliza	30	15

In comparison between wet mount and rapid test there was statistically significant difference ($p<0.05$) as the wet mount detected 10 positive cases (40%) out of 25 positive cases (100%) detected by culture (Table 2).

Table (2): Comparison between rapid test of trichomonas vaginalis and wet mount smear for diagnosing *T.vaginalis*.

Wet mount smear	Rapid test						P-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
Positive	10	40	00	00	10	5	<0.05
Negative	15	60	175	100	190	95	S
Total	25	100	175	100	200	100	

In comparison between Giemsa stain and culture there was statistically significant difference ($p<0.05$) as Giemsa stain detected 15 positive cases (60%), out of 25 positive cases (100%) detected by rapid test, there is three positive cases detected by rapid test and not by giemsa stain (Table 3).

Table (3): Comparison between rapid test and Giemsa stain for diagnosing T. vaginalis.

Giemsa stain	Rapid test						p-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
Positive	15	60	3	1.6	18	9	<0.05
Negative	10	40	172	98.2	182	92	S
Total	25	100	175	100	200	100	

In comparison between rapid test and culture there was no statistically significant difference ($p>0.05$) as rapid test detected 25 positive cases (92.5%), out of 27 positive cases (100%) detected by culture (Table 4).

Table (4): Comparison between culture and rapid test for diagnosing T. vaginalis infection.

Rapid test	Culture						p-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
Positive	25	92.5	00	00	25	12.5	>0.05
Negative	2	7.5	173	100	175	87.5	NS
Total	27	100	173	100	200	100	

In comparison between Rapid test and culture there was no statistically significant difference ($p>0.05$) as rapid test detected 25 positive cases (83.3%), out of 30 positive cases (100%) detected by ELIZA (Table 5).

Table (5): Comparison between culture and ELIZA for diagnosing T. vaginalis infection.

Rapid test	ELIZA						p-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
Positive	25	83.3	00	00	25	12.5	>0.05
Negative	5	16.7	170	100	175	87.5	NS
Total	30	100	170	100	200	100	

In comparison between ELIZA and culture there was no statistically significant difference ($p>0.05$) as culture detected 27 positive cases (90%), out of 30 positive cases (100%) detected by ELIZA (Table 6).

Table (6): Comparison between culture and ELIZA for diagnosing T.vaginalis infection.

Culture	ELIZA						p-value
	Positive		Negative		Total		
	No.	%	No.	%	No.	%	
Positive	27	90	00	00	27	13.5	>0.05
Negative	3	10	170	100	173	86.5	NS
Total	30	100	170	100	200	100	

In comparison between sensitivity, specificity, accuracy and predictive values for different vaginal trichomoniasis diagnostic techniques, ELIZA gives the highest sensitivity, specificity, accuracy, NPV and PPV (100%) followed by culture (90%). The least sensitive is wet mount (40%) and the least specific is Giemsa stain (60%) (Table 7).

Table (7): Sensitivity, specificity, accuracy, NPV and PPV of different diagnostic techniques.

Diagnostic tests	Sensitivity (%)	Specificity (%)	Accuracy (%)	NPV (%)	PPV (%)
Wet mount	40	100	92.5	92.1	100
Giemsa stain	60	98.8	93.5	94.05	83.33
Rapid test	83.5	100	98.5	98.2	100
Culture	90	100	98.5	98.2	100
ELIZA	100	100	100	100	100

The highest prevalence of vaginal trichomoniasis (30%) was recorded in suspected cases having combination of vaginal discharge, itching, dysuria, dysparounia, abdominal and back pain equal (36% of total positive), while the least prevalence (10%) was recorded in suspected cases presenting dysuria (10%) vaginal discharge only (11.5). Itching only has no value in diagnosis of trichomoniasis. The difference between data in the studied groups was statistically significant (p -value <0.05) (Table 8).

Table (8): Frequency of symptoms among studied cases.

Symptom	No. of studied cases	Positive cases of T. vaginalis		Negative cases of T. vaginalis		p-value
		No.	%	No.	%	
		• Vaginal discharge	35	4	11.4	
• Itching	40	0	0	40	100	S
• Infertility	5	0	0	4	80	
• Vaginal discharge and itching	50	5	10	45	90	
• Dysurea and dysparounia	20	3	15	18	90	
• Vaginal discharge dysurea, dysparounia, abdominal pain	50	18	36	32	64	
Total	200	30	15	170	85	

Purulent yellowish greenish frothy vaginal discharge is the most common character of *T.vaginalis* discharge (28% of cases) and the least character is watery discharge (5.7% of suspected cases). Bloody vaginal discharge has no value in diagnosis. The difference between data in the studied groups was statistically significant (p -value <0.05) (Table 9).

Table (9): Vaginal discharge characters in studied cases.

Character of vaginal discharge	Studied cases	No. of studied cases (n=200)	Positive T. vaginalis cases		Negative T. vaginalis cases		p -value
			No.	%	No.	%	
• Purulent frothy yellowish green		70	20	28	50	7.1	<0.05 S
• Whitish		40	5	12.5	35	8.7	
• Mucoid		50	3	6	47	9.4	
• Watery		35	2	5.7	33	9.4	
• Bloody		5	0	0	5	100	
Total		200	30	11.1	170	88.9	

T. vaginalis infection was most prevalent in the age groups 30-35 years (25% of suspected cases) and it was less prevalent in age ranged from 45-50 years (5% of suspected cases). The difference between data in the studied groups was statistically significant (p -value <0.05) (Table 10).

Table (10): The relation between vaginal trichomoniasis and age of studied cases.

Age range	No. of studied cases	Positive T. vaginalis cases		Negative T. vaginalis cases		p -value
		No.	%	No.	%	
20-25	30	4	13	26	86	<0.05
25-30	50	8	16	42	84	S
30-35	40	10	25	30	75	
35-40	25	5	20	20	80	
40-45	25	3	12	22	88	
45-50	20	1	5	19	95	
Total	200	30	15	170	85	

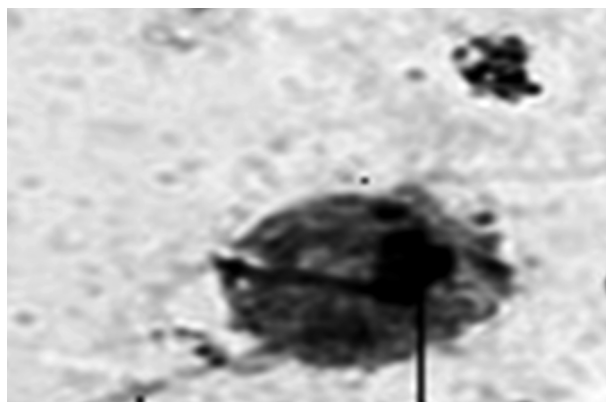


Fig. (1): Gimsa stain of Trophozoite of *Trichomonas vaginalis* showing flagalle and nucleus.

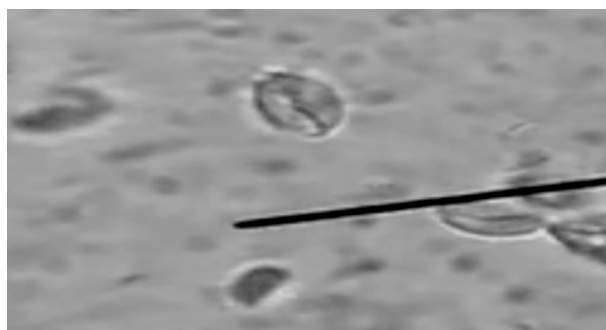


Fig. (2): Wet mount smear of Trophozoite of *Trichomonas vaginalis* showing flagalle and nucleus (X1000).

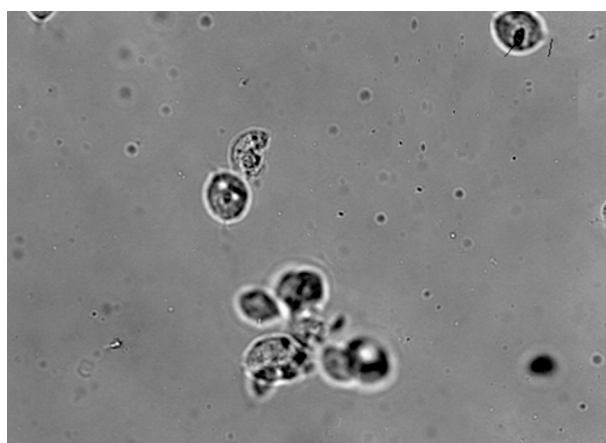


Fig. (3): Culture showing *Trichomonas vaginalis* Trophozoites with nucleus axostyle and flagella (x640).

Discussion

Trichomoniasis is the most common nonviral Sexually Transmitted Disease (STD) worldwide, although data are limited for women with a low prevalence of infect). Vaginitis due to *Trichomonas vaginalis* clinically manifests with symptoms of

vaginal itching, odor, and discharge. Recent studies also show that *T. vaginalis* is an important cause of the premature rupture of membranes, premature delivery, pelvic inflammatory disease, urethritis, and chronic prostatitis. Trichomoniasis infection also enhances the transmission of human immunodeficiency virus infection [10].

Microbiology laboratory tests a high volume of vaginal swabs each day for vaginitis pathogens, and 30% are positive for bacterial vaginosis or candidiasis, although there is a low prevalence of *T. vaginalis* vaginitis based on the detection of motile trichomonads by Wet Preparation examination (WP). Due to the poor sensitivity of Wet mount smear, *T. vaginalis* cases likely were being missed because of lost flagellate viability during specimen transport [11]. New diagnostic methods for trichomoniasis recently have become commercially available that do not require the presence of viable flagellate, including rapid antigen immunocapillary and nucleic acid amplification tests. It was therefore of interest to compare the performance of the OSOM Trichomonas rapid test (OSOM Trich) (Genzyme Diagnostics, Cambridge, MA) to that of WP for the routine detection of *T. vaginalis* vaginitis in our laboratory setting [12].

Till today, in developing countries including Egypt, little emphasis has been laid on the importance of decreasing the rates of trichomoniasis even though it has been associated with human immunodeficiency virus acquisition. Because vaginal discharge which is the major symptom of trichomoniasis is a non specific symptom, laboratory diagnosis is must for detecting the infection [13]. Although effective therapeutic agents for treatment of trichomoniasis are widely available, diagnostic difficulties have become the limiting factor in reducing the disease burden. Various laboratory methods have been employed for the detection of trichomoniasis in vaginal discharge which vary in their sensitivity and specificity [13].

Accurate diagnosis of *T. vaginalis* is affected by many variables, including patient factors, clinician's experience, specimen sampling, processing and test interpretation as well as the skill set and expertise of those doing microscopic assessments. On the other hand, sensitivity, specificity, cost, ease of use and time consuming for the different methods for diagnosing *T. vaginalis* infection should be also considered [11].

In the present study, wet mount detected 5% (10 positive cases out of total 200 suspected women). The sensitivity of wet mount was 40% as it

detected 10 positive cases out of total 27 positive cases detected by culture method. The previous results came in coordination with, Nagat et al., [14] who reported that wet mount microscopy detected about half of the culture positive samples 13 (56%) cases positive by wet mount out of 22 total positive cases and 13 cases positive (56%) by wet mount out of total 22 positive cases respectively. Schirm et al., [15] who reported 27 (67.5%) cases positive by wet mount out of total 40 positive cases by culture method and Sood et al., [16] who reported that wet mount microscopy detected about half of the culture positive samples 22 (55%) cases positive by wet mount out of 40 total positive cases and 13 cases positive (62%) by wet mount out of total 21 positive cases respectively. On the other hand, Mahmoud et al., [17] reported 12 cases positive (34.2%) by wet mount out of 35 total positive cases by culture. Huppert et al., [18] reported that wet mount alone is insufficient for the reliable diagnosis of *T. vaginalis* infection in women. Gavvani et al., [19] reported 31 cases positive (33.6%) by wet mount out of total 92 positive cases by culture.

In many health settings worldwide, wet mount microscopy is the preferred option for prompt diagnosis of trichomoniasis. This method, save time and enables patients to receive treatment in the same day and will reduce clinical over-diagnosis in women presenting with vaginal discharge [20].

Different reports indicated that the sensitivity of wet mount is between 60% when compared with the culture method [11].

On the other hand, wet mount has its short comings. The sample should be examined as soon as possible, because *T. vaginalis* is perishable in external conditions. Wet-preparation microscopy is not routinely performed, even in many STI clinic settings and laboratories [21].

Giemsa staining method detected 7.5% (15 positive cases out of total 200 suspected cases) The sensitivity of Giemsa stained method was 60% compared to culture method as it detected 15 positive cases, compared to 27cases positive by culture and 25 positive cases detected by rapid test. In coordination with Nagat et al., [14] who reported that 15 (60%) cases were positive by Giemsa staining method compared to total 27 (83.5%) positive cases by culture. On the Ojuromi et al., [22] who reported that 62 (73%) cases were positive by Giemsa staining method compared to total 85 (100%) positive cases by culture. On the other hand Radonjic et al., [5] reported that 11 (50%) of cases were positive by Giemsa stain out of total

22 (100%) cases positive by culture method with 50% sensitivity of Giemsa stain compared to culture method and El Sayed Zaki et al., [23] reported 16 (48.5%) positive cases by Giemsa stain compared to total 33 (100%) positive cases by culture.

In the present study, it is observed that wet mount examination, culture and rapid test and ELIZA had 100% specificity; this could be attributed to the fact that the presence of Trichomonads was determined by its characteristic size, shape and mobility, this what explained by Sood et al., [16]. However, Giemsa staining diagnosed five positive cases which were found to be negative by both wet mount examination and culture. These could be considered false positives as diagnosis by the latter two methods depends not only on size and shape but also on mobility of the parasite.

Rapid test, used for diagnosis of trichomoniasis and is low cost and time consuming [24]. OSOM Trich is an immunochromatographic capillary-flow enzyme immunoassay dipstick test that was performed according to the manufacturer's instructions. Vaginal swabs were tested in batches of 10, and analysis required 10 to 15min. A positive result has both a red internal control and a blue positive test line, while the negative result has only a red internal control line. Invalid tests had an absent internal control line. Discrepant results between OSOM Trich and WP were resolved using the Aptima Trichomonas transcription-mediated amplification assay performed according to the manufacturer's instructions (Gen-Probe, San Diego, CA). Specimens were tested in a Gen-Probe DTS 400 instrumentation system using *T. vaginalis*-specific reagents and Aptima general-purpose reagents (target capture, transcription-mediated amplification, and hybridization protection using primers and probes that specifically target *T. vaginalis* rRNA). A *T. vaginalis* patient culture in Diamonds medium was used to inoculate a Gen-Probe Aptima swab specimen transport tube and an uninoculated Gen-Probe Aptima swab specimen transport tube, which were used as positive and negative controls [11].

In the present study, rapid test detected 12.5% (25 positive cases out of total 200 suspected women). The sensitivity of wet mount was 90% as it detected 25 positive cases out of total 27 positive cases detected by culture method. The previous results came in coordination with, [25] who confirms that OSOM Trich had improved sensitivity (90.7%) compared to that of WP (60%) in this context. OSOM Trich also had the excellent specificity required for screening patients with a low preva-

lence of infection. Huppert et al. [5,6] recently evaluated OSOM Trich in two separate studies in female populations with a high prevalence of *T. vaginalis* infection. The initial evaluation used OSOM Trich to rapidly detect *T. vaginalis* in vaginal specimens collected from sexually active women 18 years of age (n 449) presenting with symptoms of vaginitis, exposure to *T. vaginalis*, or multiple sexual partners. Their study population had a high prevalence of *T. vaginalis* at 23.4%. OSOM Trich detected more *T. vaginalis* cases, with a sensitivity of 83.3%; that for WP was 71.4%. In a more recent study, sexually active adolescent women aged 14 to 21 years (n 330) were recruited from a teen health center and the emergency department). Vaginal swabs were tested for *T. vaginalis* using WP, culture (in Pouch *T. vaginalis*; BioMed Diagnostics), OSOM Trich, and Aptima. Their study group also had a high prevalence of trichomoniasis at 18.5%. The sensitivity of each method was compared to that of a CPS (i.e., any test with positive results). WP had the lowest sensitivity (56%), while Aptima had the highest sensitivity (98.4%). Although OSOM Trich (83%) had lower sensitivity than that of culture (90%), test results were available the same day, whereas it takes a several days' delay for culture.

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دراسات طفيلية على طفيل التريكيوموناس المهبلية لدى السيدات المصابات بالإفرازات المهبلية بمحافظة أسوان

أجريت هذه الدراسة على ٢٠٠ من النساء المترددات على عيادة النساء والتوليد بمستشفى أسوان الجامعى اللاتى يعانين من مختلف شكوى أمراض النساء، اللاتى تتراوح أعمارهن بين ٢٠-٥٠ سنة. تم أخذ مسحتين مهبليتين من كل مريضة بعد أخذ موافقتها.

طرق البحث:

- الفحص المجهري المباشر لعينة المسحة المهبلية.
- الفحص بإستخدام صبغة (لطفة) الجيمسا.
- الفحص بإستخدام الإختبار السريع الأنتيجينى.
- الزرع لعينات المسحة المهبلية على مزرعة دايموند المعدلة. وتأثير بعض الأعشاب والأدوية عليه.
- الإليزا.

أظهرت النتائج أن الزرع لعينات المسحة المهبلية على مزرعة دايموند المعدلة بينت أفضل النتائج حيث كان معدل الإصابة (١٥٪). وإستخدام الإختبار السريع الأنتيجينى كان (١٢.٥٪) وإستخدام صبغة الجيمسا (٧.٥٪) وأقلها بإستخدام الفحص المجهري المباشر (٦٪)، وبالتالي فإن معدل إنتشار مرض التريكوموناس فالجيناليس فى هذه الدراسة هو (١٥٪). وإستخدام الإليزا كان المعدل (١٥٪).

وأظهرت النتائج أن الزرع لعينات المسحة المهبلية على مزرعة دايموند المعدلة يعطى أعلى حساسية، خصوصية، ودقة، وقيمة تنبؤية إيجابية وقيمة تنبؤية سلبية، تليها الإختبار السريع، أما الأقل حساسية هو الفحص المجهري المباشر بينما الأقل خصوصية والأقل قيمة تنبؤية إيجابية هو صبغة الجيمسا.

هناك فروق ذات دلالة إحصائية فى معدل الإصابة بمرض التريكوموناس فالجيناليس مع تصنيف الحالات المشتبه فيها وفقاً لتواتر الأعراض حيث تبين من البحث أن معدل الإصابة بمرض التريكوموناس فالجيناليس يرتفع فى المرضى اللاتى يعانين من مزيج من الأعراض (إفرازات مهبلية - حكة - عسر التبول - عسر الجماع - ألم فى الظهر والبطن)، بينما ينخفض معدل الإصابة فى المرضى اللاتى يعانين من إفرازات مهبلية فقط.