



ISSN: 2789-1089 EISSN: 2789-1097

NTU Journal of Pure Sciences

Available online at: <https://journals.ntu.edu.iq/index.php/NTU-JPS/index>



Evaluation of direct microscopy and culture method for detection of *Trichomonas vaginalis* in vaginal discharge and urine samples

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Article Informations

Received: 23-10-2022,
Accepted: 10-11-2022,
Published online: 15-03-2023

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Key Words:

Microscopy,
Culture,
Trichomonas vaginalis,
Vaginal discharge,
Urine.

ABSTRACT

Trichomoniasis has emerged as the most common sexually transmitted disease, and limited data is available on the effective screening technique for the diagnosis of *Trichomonas vaginalis* (*T. vaginalis*). This study aimed to compare the ability of two culture media (InPouch *TV* and Diamond's) to support the growth of clinical isolates of *Trichomonas vaginalis* and their relative sensitivity for detection of the organism. A total of 343 patients complaining of vaginal discharge of 293 women and urine sample of 50 men were included in the study. From December 2021 to May 2022, from Azadi Teaching Hospital, Private clinics, and Midwifery and Childbearing Hospital in Kirkuk. Three vaginal swabs and a urine sample were screened for trichomoniasis by wet mount microscopy. Diamond Media Culture and Pouch *TV* were used. The 343 cases studied, 6 women and 1 man were positive by wet mount microscopy, which means 7 (2%) and 336 (98%) were negative. Sterile vaginal swab with centrifugation and urine samples had the highest rate of specificity (85%) to detect *TV* compared to vaginal swab without centrifugation and Amies Gel Transport Media (AGT) media, which had a lower sensitivity (33% and 16%), respectively. Inpouch *TV* media was the best culture to grow *TV* and remained for 10 days, but Local Diamond Modified Media (LDMM) had a poor result for growth of *trichomonas vaginalis* and only remained alive for 2 days. According to our experiment, the most successful routes of detection are urine and vaginal swab with centrifugation, and Inpouch *TV* is the unique culture for growth and better than LDMM.



Introduction

The parasitic protozoan *Trichomonas vaginalis* causes trichomoniasis, a sexually transmitted disease. Therefore, with an estimated 170 million cases worldwide each year, it is considered as the most common non-viral sexually transmitted disease [1]. It is spread by sexual activity [2, 3], non-venereal methods such as sharing infected towels or underwear, and the use of non-sterile medical examination tools [2, 3]. It causes vaginitis and cystitis in women and urethritis and prostatitis in men [4]. In women, the infection of *T. vaginalis* are highly asymptomatic [5], and the proportion in men could be even higher. Microscopic examination of wet mount preparations, which has a sensitivity of about 60%, is still the most commonly used method for diagnosing *T. vaginalis* infection [6]. Microscopic examination of parasite cultures in specific media increases sensitivity to 85 to 95 percent [7, 8]. Wet mount microscopy is a simple, quick, and economical technology that only requires a microscope and skilled workers. However, its sensitivity is minimal when compared to culture. It is extremely reliant on microscopes expertise, fast transit (cannot be held for an extended period of time), and laboratory processing of the sample before the organisms lose motility or become nonviable [9, 10]. This approach is less sensitive than culture. The sensitivity of wet mount is 60%, and the sensitivity of culture is 73.3% (11). Diamond modified culture detected the highest rate of *T. vaginalis* infection [12]. The most common and quick procedure for diagnosing trichomoniasis is a direct microscopic examination of vaginal secretions. The most sensitive, but slowest, diagnostic approach is vaginal and urethral specimen culture [8, 13]. Diamond's test was substantially more sensitive than the InPouch TV test [13]. Because of the limitations of microscopy, the goal of this study was to examine four sample collection methods to see which one produced the best results (swab with centrifuge, swab without centrifuge, AGTM, urine). In vitro growth of *T. vaginalis* was also compared using LDMM and InPouch TV media.

Material and methods:

Collect the samples from 293 female and 50 male patients in the age groups of 200 patients at 18–40 years old and 143 at 41–70 years old. 150 patients live in the city center and 193 in its environs. Also the different levels of knowledge, 180 educated and 183 illiterate.

Specimen collection

Three vaginal swabs (cotton tips) from the posterior fornix of the vagina and urine samples were obtained from suspected women who attended Azadi Teaching Hospital, private clinics, midwifery and childbearing hospitals from the period of December 2021 to May 2022. Clinical signs and symptoms were recorded, including discharge, pruritus, and odor. A questionnaire form (age, residential area, odor, color of discharge and itching) was also recorded for each woman, Table.1. The samples were examined as follows:

1. A drop of normal saline was used to prepare a wet mount preparation on a glass slide. The slide was first scanned at 100X to look for motile trichomonads, and then at 400X to confirm motility, flagellar movement, and the organisms' morphologic properties. Wet mounts that were negative were evaluated for at least two minutes [14].
2. An Amies gel transfer system (Zahra Al-Rawan.) was used to make the second swab [6]. Swabs were kept at room temperature for 24 hours and then microscopically tested for motile trichomonas [10].
3. To elute the material, the third swab was placed in 3.0 ml of normal saline, agitated, and pressed against the tube's wall. The vaginal specimens were centrifuged at 15,000 rpm.
4. The patient was also asked to contribute 20 mL of urine, which was decanted after being pelleted at 1,500 g for 5 minutes. To protect the viability of the trichomonas for microscopic study and culture, they were first centrifuged at a low speed [15].

Culture Media preparation

Diamond medium: Although Diamond's medium modified is a commercial product, we were able to source it locally in Bagdad (al-Bashir Scientific Office). The medium was given a 26-week shelf life from the date of production. 16.8 g of powder media components (gms/liter) were dissolved to make this. double-distilled water to 100.0 ml, the solution was filtered with Whatman no. 1 paper before autoclaving it for 15 minutes at 121°C and 15 lb/in² pressure) [16]. Then 10 mL of sterile inactivated horse serum, 1 ml of Nystatin solution, 1 ml of penicillin, 500µl of streptomycin, and vancomycin were added to the medium to inhibit bacterial and fungal growth. Before inoculation, 5 ml of the media were placed in glass tubes (10-ml volumes into 16-by-125-mm screw-cap tubes). To ensure sterility, the samples from the tubes were incubated for two days at 35–37 °C (Fig.1). After that, the diamond-modified medium was held at 4-8°C and brought to room temperature before use [7, 8, 17].

InPouch TV media is a self-contained media device for detecting and recovering *T. vaginalis* from female vaginal samples or male urethra/urine samples. The proprietary medium is selective for *T. vaginalis* transport and multiplication while preventing the growth of other bacteria, which can make a definitive diagnosis difficult (kit instruction). InPouch TV is a soft plastic double-pouched container made of a high-barrier, oxygen-resistant plastic with two V-shaped chambers connected by a tiny passage that offers a variety of advantages when used together. Un inoculated pouches should be stored horizontally at 18–25°C, away from direct sunlight, and never refrigerated or frozen. InPouch products have a shelf life of 12 months from the date of manufacturing. A clear, amber liquid should be present in an unopened packet. The final pH of the medium is 6.1 ± 0.05 . (Assembly instructions) The Inpouch medium for TV cultivation was imported from the United States (manufactured by Biomed Diagnostics).

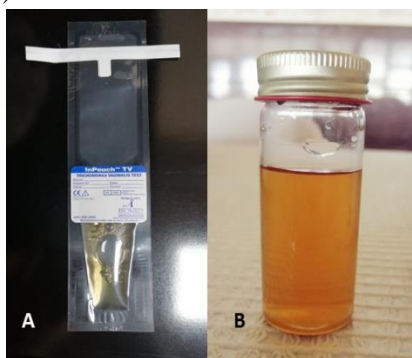


Figure 1: Used culture media, A (InPouch TV Media), B (Diamond Modified Medium).

Inoculation and Incubation

Diamonds Medium: Swabs or urine were inoculated in the culture media as soon as possible after collection. Modified Diamonds Medium was inoculated with swabs or urine. The swab inoculation was done by immersing the swab specimen in the medium, and gently twirling. 50µl of sediment urine was cultured in the media as well. The cultures were incubated at 30-35°C. for 1-3 days and daily checked for motile trichomonas by microscopic; examination of a drop of culture under 100x-400x magnification. The result was recorded (kit instruction).

InPouch TV Media: To avoid fluid leakage, the fluid was pressed from the top of the pouch downward into the bottom chamber during the production of InPouch. Above the white clasp, the plastic top has been ripped off. The pouch is opened, and 50 micro liters of urine is introduced, or the swab is kneaded between the pouch walls. Inoculated at 35-37°C for 10 days and monitored daily by microscopic examination. either by placing the InPouch directly on the microscope stage for low power (100x) observation or by withdrawing a little drop of the broth under 100x-400x.

Calculating the percentage of TV growth in cultural media

15-20µl of culture liquid was placed on a hemocytometer chamber to estimate and track the quantity of parasites in the culture media [18]. The number of the organisms per each milliliter of the suspension were counted using the this equation:

$$\text{Parasites number} = \frac{\text{Number of counted trichomonads in all squares} \times 10^4}{\text{Counted squares}}$$

Statistical analysis

The results were statistically analyzed by applying the mini-tab statistical program and by the Chi square and F-test. The arithmetic means were compared to determine significant differences according to the duncun test at the probability level of 0.05 and 0.01.

Results

According to the obtained data, most of the studied factors were significantly related to *T. vaginalis* distribution. For instance, except for one, all of the seven positive cases (six females and one male) were under the age of 40. The vast majority of the patients—five positive samples were from the environs and two from the city center. The level of knowledge was not significantly different between educated and uneducated patients suffering from itching with a fishy odor and yellow to greenish discharge, which were highly significantly associated with trichomoniasis. However, blood in the discharge were not significant. Table 1.

Table 1: Factors related to *T. vaginalis* prevalence

Factors	Patients (%)	Positive TV (%)	Chi-Square (P-Value) *, ** = significant ns = non-significant
Age:			
18-40	200 (58.3)	6 (3)	
41-70	143 (41.7)	1 (0.29)	2.728 (0.045) *
Gender:			
Male	50 (14)	1 (2)	
Female	293 (85.4)	6 (2.04)	0.0003 (0.983) Ns
Residence area:			
City center	150 (43.7)	2 (1.33)	
Environs	193 (56.2)	5 (2.59)	0.642 (0.423) ns
Level of knowledge			
Educated	180 (52.4)	2 (1.11)	
Illiterate	163 (47.5)	5 (3.06)	1.571 (0.210) ns
Odor of discharge:			
Smelly	43 (12.5)	7 (16.2)	
Odorless	300 (87.4)	0 (0.00)	42.857 (0.0009) **

Color of discharge:			
Milky/white	316 (92.1)	2 (0.63)	
Yellow/green	27 (7.87)	4 (14.8)	25.186 (0.0008) **
Blood in discharge:			
Bloody	65 (18.9)	1 (1.53)	
Bloodless	278 (81)	6 (2.15)	0.098 (0.755) ns
Suffer from itching:			
Have itching	55 (16)	6 (10.9)	
Not have itching	288 (83.9)	1 0.340	23.144 (0.0007) **

As indicated in table 2, out of the 343 women enrolled, seven samples were positive. However it reflected the low occurrence of the parasite among the population of Kirkuk city, which was (7 (2.04 %)). This was offset by high percentage (336 (97.9%)) of non-infected people.

Table 2: *T. vaginalis* prevalence with percentage.

Patients	Samples no.	Percentage (%)
Total no. examined	343	100
Positive samples	7	2.04
Negative samples	336	97.9

The result of the comparison between specimen collection and its outcomes depended on the sensitivity and specificity of direct microscopy by three types of vaginal swabs and urine, with the number of true positive and negative, also false positive and negative, were presented in Table 3, (fig. 2) indicates *T. vaginalis* trophozoites. The best samples for the detection of *T. vaginalis* were 3ml saline vaginal swabs and 10ml of urine samples. They had a sensitivity of 83%, 85% respectively and specificity of 100%. Following that sterile swab stick and Amies gel transport media, these two routes are not very sensitive at 33% and 17% respectively, (Fig.3.) But when the swab is put on 3ml of normal saline then centrifuged for 5 minutes, it is elevated to 85%.

Table 3: Comparison of used methods for *T. vaginalis* diagnosis

Type of Sample	No. true +ve	No. false +ve	No. true -ve	No. false -ve
Vaginal Swab	2	0	289	4
Vaginal Swab (centrifugation)	5	0	392	1
Amies Gel Transport Media	1	0	288	5
Urine	6	0	335	1
Chi-square (P-Value)		10.093 (0.021) *		



Figure 2: Wet mount *T. vaginalis*: right giemsa stained 1000X and left unstained 1000X (A- Anterior Flagella, B- Undulating membrane, C- Axostyle)

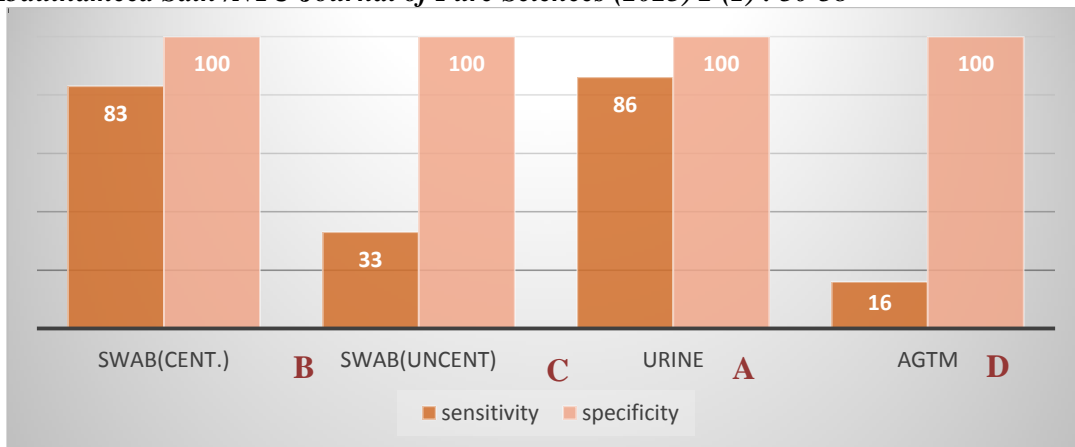


Figure 3: Sensitivity and specificity of used diagnostic methods, (The letters refer to the degree of significance, A have the highest significant and D the lowest).

Cent.- Centrifuge

Uncent.- Uncentrifuge

The two standard culture media used for the growth of the protozoa which was Diamond's modified and Inpouch TV media. Trichomonas growth in each medium is mentioned in (Fig.4.) TV was inoculated in both media and monitored to distinguish the number and viability of the organism. After the initial inoculation of 50 μ l (30×10^4 orgs /ml) into each culture medium, the detected number of organisms in LDMM was (11, 5, 0) $\times 10^4$ orgs /ml after 24, 48, 72 hrs. A small number of the organisms remained alive for two days, then all died. However, in InPouch TV media, the growth of TV was (55, 100, 190, 194, 194, 194) $\times 10^4$ orgs /ml throughout 24, 48, 72, 96, 120, 144 hrs. after inoculation. However the trophozoite remained alive for 10 days. The InPouch TV test demonstrated greater sensitivity than Diamond's media, (Fig. 5) shows the parasite on counting chamber.

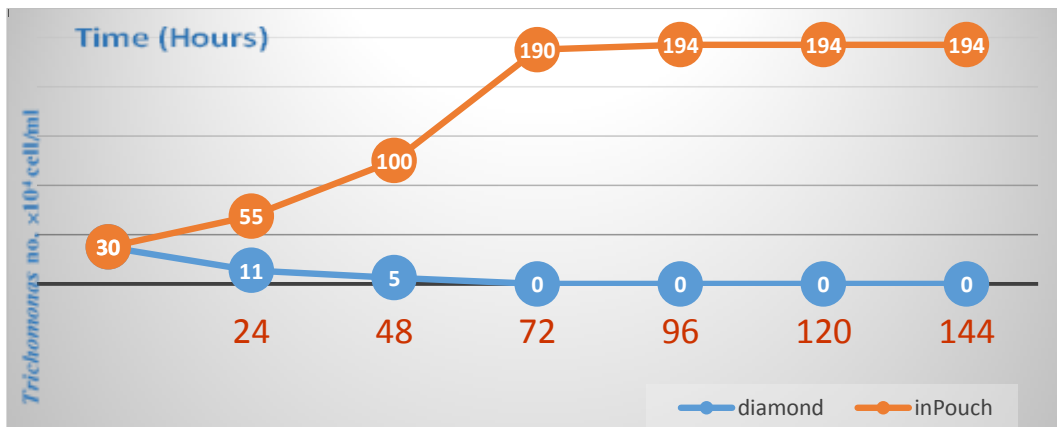


Figure 4: Growth of *T. vaginalis* in LDMM and InPouch TV Media.

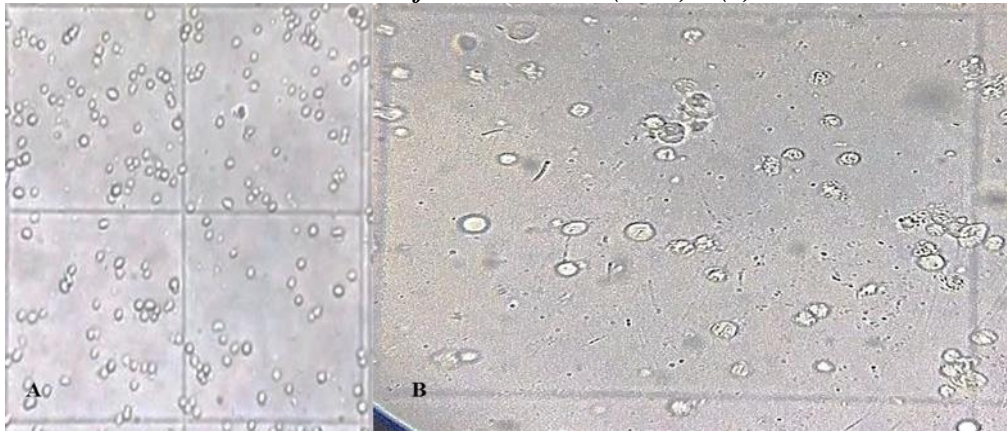


Figure 5: *T. vaginalis* on counting chamber. A (unstained smear10X), B (unstained smear 40X).

Discussion:

The prevalence of *Trichomonas vaginalis* in the present study was 3 that was somehow agree with United States, which is 2.1 % among women aged 14 to 59, and disagree with 9.6 % among African American women (19). Between 2002 and 2020, 65 articles about *T. vaginalis* infection in the general Turkish population were found. Infection was found to be prevalent in 5.94% and 6.17% of men and 2.87% of women (20); that was in disagreement with the current study, in which no-significant difference between the rate of infection was 2% for men and 2.04% for women.

Furthermore, there was no statistically significant difference between people who live in the city center and those who live in its surroundings; their rates were 1.33% and 2.59, respectively. As agreed with our study, when infection was analyzed in relation to education, no significant differences were discovered (21).

The symptoms in (Patient Care & Health Information) and (Sexual Health), as in our study questionnaire, stated that trichomoniasis can cause a foul-smelling vaginal discharge, genital itching, and painful urination. An often foul-smelling vaginal discharge, which might be white, gray, yellow, or green, genital redness, burning and itching, pain with urination or sexual intercourse (14, 22, 23). Significantly more people under the age of 40 had a fishy odor, a yellow to green color, and itching than people over the age of 40, who had no odor, a white color, and no itching. However, there were no significant differences between the genders, location, education, blood, cyclic periods, and thyroid diseases compared with others who had not.

The World Health Organization estimates that there are approximately 220 million instances of TV infection worldwide (19). The infection rate in our study sample was 2.04%. This is the same as and lower than the infection rate given by the Centers for Disease Control and Prevention 2.1% (24).

Furthermore, urine and centrifuged swabs detected *T. vaginalis* with the highest significant sensitivity (85% and 83%) respectively, with no significant difference from (Hsin-Yao Wang, Chung-Chih Hung, and others) who reported urine sensitivity of 83.5% (25). In contrast to (A. L. Beverly, M. Venglarik, etc.), who reported a sensitivity of 91.2% for Amies gel transport media, AGTM has the lowest significant range (16%) (26). According to (K A Borchardt 1, M Z Zhang, et al), the InPouch TV test revealed more sensitivity than Diamond's media (17) like our findings. When compared to Diamond's modified medium culture, InPouch has some distinct advantages. Once the specimen is placed in the InPouch chamber by a clinician, microscopic inspection can be done immediately through the bag without the need for a sample to check for growth. The InPouch also gives clinicians a quick way to get specimen and culture results. Additionally, the former can be kept at room temperature for up to a year. Although both tests take the same amount of time for a technician to complete. The results are vastly different since the Inpouch is more precise and reliable. Diamond's modified medium has a reduced shelf life and must be kept refrigerated. However, as compared to a tube of Diamond's medium, the InPouch is more expensive.

Conclusions:

The InPouch TV test was far more sensitive than Diamond's media, which develops trichomonas for four days and then leaves them for ten days. The Local Diamond, on the other hand, barely lasts two days before the protozoa begin to multiply. Furthermore, we exhibited the ability of dry swabs and AGTM did not have a satisfactory result for detection (33% and 16%, respectively), in comparison to urine and centrifuged swabs with a sensitivity of 85%.

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