

**COMPARATIVE ANALYSIS OF DIAGNOSTIC METHODS IN THE DETECTION OF
TRICHOMONAS VAGINALIS INFECTIONS**

ABSTRACT

Background:

Trichomonas vaginalis is a flagellated protozoan known to cause sexually transmitted infection across sexes but women tend to be symptomatic thereby leading to premature labour, low birth weight, and post-abortion or post-hysterectomy infection among pregnant women. The organism demonstrates a wobbling and rotating motion under the microscope. The aim of this study was to determine the validity of the Giemsa staining technique and Direct wet mount technique for the diagnosis of *Trichomonas vaginalis* infection against the gold standard culture method.

Materials and Methodology:

The study was a cross-sectional study of two hundred (200) sexually active females in Internally Displaced Persons (IDP) camps who presented to the clinics with vaginal discharge. Clients were recruited consecutively until the number required was obtained from each camp. High vaginal swab samples were collected and processed using Giemsa staining, Direct wet mount, and Culture in OXOID *Trichomonas* broth. Statistical Package for Social Sciences (SPSS) version 21 was used for statistical analysis.

Results:

Out of 200 samples examined, the culture method identified 41 positive samples. Giemsa staining method had 32 true positives with 9 false negatives and 3 false positives as compared to the culture method. Conversely, the Direct wet mount technique observed 33 positives out of which 28 were true positives and 5 false positives as they were negative by the Culture method. Giemsa staining technique had a sensitivity of 78.1% and specificity of 98.2%. The positive and negative predictive values of the Giemsa staining were 91.4% and 94.6% respectively. The Direct wet mount technique had a sensitivity of 68.3%, specificity of 97.0%, positive predictive value of 84.9%, and

negative predictive value of 68.3%.

Conclusion:

The findings of this study showed that Giemsa staining technique performed well and showed a high sensitivity than the Direct wet mount in the detection of *Trichomonas vaginalis* and as such recommended for use in absence of the culture method.

Keywords: Culture, Direct wet mount, Giemsa staining, IDP Camps, *Trichomonas vaginalis*

INTRODUCTION

Trichomonas vaginalis is an etiologic agent of the disease trichomoniasis. It is a flagellated protozoan with four free flagella and a free flagellum attached to the surface of the parasite to form an undulated membrane ^[1]. Trichomoniasis is a sexually transmitted infection, which can occur in females if the normal acidity of the vagina is shifted from a healthy, acidic pH (3.8-4.2) to a much more semi-acid (5.0-6.0) conducive to *Trichomonas vaginalis* growth ^[2]. The symptoms associated with the clinical diagnosis of *T. vaginalis* include a yellowish-green frothy discharge, pruritus, dysuria, and dyspareunia among others.

Diagnosis of trichomoniasis has traditionally depended on the microscopic observation of motile trophozoites of *Trichomonas vaginalis* in vaginal secretions or urine. The detection of the parasite is made possible by examination of urine deposit and high vaginal swab (HVS) in a drop of saline or trichomonas diluents for the characteristic wobbling and rotating motion of *T. vaginalis* ^[3]. The

Giemsa stain, Leishman, Periodic Acid Schiff, Polymerase Chain Reaction, Fluorescent microscopy, Direct wet mount, and Culture, are some of the available diagnostic methods for trichomoniasis^[4]. The broth culture method is the “gold standard” for the diagnosis of trichomoniasis because it is simple to interpret and requires as few as 300 to 500 trichomonads/ml of inoculum to initiate growth in culture ^[5].

Most laboratories in developing countries still depends on the Giemsa stain and Direct wet mount to establish the diagnosis of trichomoniasis as the culture method is not routinely available. However, the validity of these methods is not extensively studied in most of these countries. Many physicians are still unclear about which of these available tests is more sensitive^[6]. There this study aimed at determining the validity of Giemsa stain and Direct wet mount for diagnosis of trichomoniasis using Culture as the gold standard.

MATERIALS AND METHODS

Study Area

The study was carried out in some selected IDP camps in Maiduguri, the state capital of Borno State, Nigeria. The city with a population of about 1,112,449 inhabitants, occupies an area of 50,778Km² (square Kilometer). It shares borders with Yobe, Adamawa and Gombe states to the north-west and south respectively.

The state with a total population of 4 151 193 (Federal Office of Statistics, 2006) has been ravaged by years of Boko-Haram terrorism which have displaced people to different IDP camps within the state capital. The four IDP camps visited for recruitment of participants have a total population of 57, 870 comprising of people of both sexes and different age group. They depend on government and Non-Governmental Organizations (NGO) for their daily feeding, clothing, hospital care and other daily requirements within the camps. The people are of different ethnic groups mainly

Kanuri, Marghi, Bura (Babur), Mandara, Shuwa Arab, Fulani, and Hausa. The people value their rich cultures and norms and still carry out some petty trading within and outside the camps.

Study population:

The study population included sexually active females living within Internally Displaced Persons Camps in Maiduguri, Borno state presenting with vaginal discharge and consented to participant in the research.

Study design:

This was a cross-sectional study of two hundred (200) sexually active females in Internally Displaced Persons Camps who present to the clinics with complaints of vaginal discharge.

Ethical Consideration:

Ethical approval was obtained from State Ministry of Health Maiduguri, Borno State. Permission was also obtained from Headquarters of 7 Division Nigeria Army Maimalari Contonment, Maiduguri; National Emergency Management Agency (NEMA), and the State Emergency Management Agencies (SEMA). Informed consents were sought from all the participants before sample collection.

Sample Collection:

Urine and vaginal swabs were carefully and aseptically collected using sterile universal container and sterile swab respectively from 200 females by a qualified Medical Practitioner after given appropriate instructions on how the sample should be collected ^[7].

Wet Mount Microscopy:

The 0.9% normal saline was added to high vagina swab container and mixed gently ^[8] and a drop

was placed on a 22 × 40 glass slide and covered with a cover slip. The preparation was examined for the wobbling motility of the trophozoites of *T. vaginalis* under the microscope using ×10 and ×40 objectives ^[9].

Giemsa' Staining Technique:

Smear slides were dried and fixed with methanol for 30 seconds and then stained with Giemsa's stain and allowed for 15 to 20 minutes at room temperature ^[10]. The slides were then rinsed with distilled water and allowed to air dry at room temperature. A drop of immersion oil was applied to the slide and viewed under x100 objective.

Culture Method:

The vaginal swab specimens were inoculated in OXOID Trichomonas medium which was prepared by using 37.5g of the medium powder in 1 litre of distilled water. The medium was allowed to boil in water bath to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. The horse serum was inactivated at 56°C for 30 minutes; the pH 6.0 was obtained using dilute hydrochloric acid (HCL) and added to the medium after cooling for 50°C. Thereafter, 80mls of horse serum for enrichment, 10mls of Oxoid penicillin-streptomycin for suppressing bacterial growth and 10 ml of Nystatin Solution for fungi growth suppression were added to 1000 ml of the media. An aliquot of about 4 ml medium was then aseptically poured into sterilized Bijou bottles, which was then stored in the refrigerator at 4°C. The specimens were inoculated on the medium immediately after collection by cutting the tip of the swab sticks into the medium, which were also gently rolled. The inoculated medium in Bijou bottles were labeled and incubated at 37°C. Finally, microscopic examination was done at intervals of 24, 48 and 72 hours under x10 and x40 magnification, which revealed the jerky motion of *Trichomonads*.

Statistical Analysis:

Data generated from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 21 (IBM SPSS Inc, USA). The proportions were compared using Chi-square at p-value < 0.05 considered significant.

RESULTS:

Out of the 200 participants screened, forty-one (41) were positives trichomoniasis using the culture method see . Giemsa staining method identified thirty-five (35) positives out of which three (3) were false positives as compared to the Culture method (Table 1). Thirty-three (33) of the 200 participants were positive using Direct wet mount. Out of the thirty-three (33) positives by direct wet mount, twenty-eight (28) were true positives and five (5) were false positives as they were negative by Culture method (Table 2). The Giemsa staining technique had sensitivity of 78.1% and specificity of 98.2%, with positive and negative predictive values of 91.4% and 94.6% respectively (Table 3). The sensitivity and specificity of the Direct wet mount technique was 68.3% and 97.0% respective with a positive predictive value of 84.9% and negative predictive value of 68.3% (Table 3).

Table 1. Correlation of Giemsa's Staining Technique and Culture method

Giemsa' stain	Culture method		Total
	Positive	Negative	
Positive	32	3	35
Negative	9	156	165
Total	41	159	200

Table 2. Correlation of Wet mount Technique and Culture method

Wet mount	Culture method		Total
	Positive	Negative	
Positive	28	5	33
Negative	13	154	167
Total	41	159	200

Table 3. Comparison of the sensitivity, specificity, positive and negative predictive values of Giemsa stain and Direct wet mount in the diagnosis of trichomoniasis.

Diagnostic methods	Sensitivity	Specificity	Predictive values	
			Positive (%)	Negative (%)
Giemsa's stain	78.1	98.2	91.4	94.6
Wet mount	68.3	97.0	84.9	68.3

DISCUSSION

Trichomonas vaginalis is one of the cause of sexually transmitted infection across the globe where patients presents with profuse yellowish vaginal discharge and frothy, malodor, dysuria and itching. Diagnosis of trichomoniasis has relied mostly on wet mount demonstration and staining of the parasite in the laboratory, various success ranging from 20-80% of this method is documented ^[11]. However, a combination of broth culture method with microscopic wet mount demonstration is now the acceptable procedure for effective diagnosis of this protozoa infection ^[12]. Although direct microscopy is easily performed and is economical, it has low sensitivity and it is affected by inoculum size and experienced of the microscopist.

The organism can be detected in vaginal, urethral and prostatic secretions as well as in semen and urine. A commonly applied diagnostic method for diagnosis of *T. vaginalis* infection in women is

microscopic examination of a wet mount preparation of vaginal secretions mixed with normal saline ^[13]. Some studies reported that culture is the gold standard for the detection of *T. vaginalis* compare to direct wet mount and Giemsa's staining technique ^{[13];[14]}. Direct examination of wet mount preparation of clinical specimen is the most rapid and less expensive technique for identifying *T. vaginalis* ^[15]. Because of the limitations of wet mount, culture remains the most accurate method for detecting *Trichomonas* infection.

This study examined the sensitivity and specificity of Giemsa's staining technique and Direct wet mount comparing with culture method been a gold standard and it was found out that Giemsa's staining technique is more sensitive and specific (78.1% and 98.2%) in detecting *T. vaginalis* than wet mount technique (sensitivity 68.3%, specificity 97.0%). Similarly, ^{[11][16]} wet mount though highly specific and rapid offers poor sensitivity (51% - 65%), detecting only half of all culture-positive cases been gold standard with sensitivity of 75-96%. In previous studies on the accuracy of the culture in the diagnosis of *T. vaginalis*, the reported specificity ranged from up to 100% and false-positive results were also describe. The study showed that Giemsa's staining technique is more sensitive and specific compare to Direct wet mount and as such recommended for use in absent of culture method.

CONCLUSION

It was concluded based on the findings of the study that Giemsa staining technique was more sensitive compare to wet mount technique in the detection of *Trichomonas vaginalis* and as such recommended for use in absent of culture method. High vaginal swab samples should be encourage to urine. Sensitivity and specificity are important measures of the diagnostic accuracy of a test but cannot be used to estimate the probability of disease in an individual patient. Positive and negative predictive values provide estimates of probability of disease but both parameters vary according

to disease prevalence.

CONSENT

All the authors reviewed and gave their consent for this article to be submitted for publication.

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APPENDIX

Parameter	Giemsa stain	Wet mount
True positives (TP)	32	28
False positives (FP)	3	5
False negatives (FN)	9	13
True negatives (TN)	159	159

Tested Statistic	Formula
Sensitivity	$(TP/TP + FN) \times 100$
Specificity	$(TN/TN + FP) \times 100$
Positive	

Predictive value	$(TP/TP + FP) \times 100$
Negative Predictive value	$(TN/TN + FN) \times 100$