Tropical Biomedicine 32(2): 1–6 (2015)

Validation of wet mount microscopy against *Trichomonas* culture among women of reproductive age group in Western province, Sri Lanka

Banneheke, H.[1,2*], Fernandopulle, R.[3], Gunasekara, U.[4], Barua, A.[5], Fernando, N.[6] and Wickremasinghe, R.[1]

[1]Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

[2]Division of Pathology, School of Medicine, International Medical University, Kuala Lumpur, Malaysia

[3]Department of Gynaecology and Obstetrics, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

[4]Medical Officer of Health Office, Nugegoda, Sri Lanka

[5]Department of Community Medicine, School of Medicine, International Medical University, Kuala Lumpur, Malaysia

[6]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

*Corresponding author email: hasini.banneheke@gmail.com

Received 8 August 2014; received in revised form 25 August 2014; accepted 5 September 2014

Abstract. Wet mount microscopy is the most commonly used diagnostic method for trichomoniasis in clinical diagnostic services all over the world including Sri Lanka due to its availability, simplicity and is relatively inexpensive. However, *Trichomonas* culture and PCR are the gold standard tests. Unfortunately, neither the culture nor PCR is available for the diagnosis of trichomoniasis in Sri Lanka. Thus, it is important to validate the wet mount microscopy as it is the only available diagnostic test and has not been validated to date in Sri Lanka. The objective was to evaluate the validity and reliability of wet mount microscopy against gold standard *Trichomonas* culture among clinic based population of reproductive age group women in Western province, Sri Lanka. Women attending hospital and institutional based clinics were enrolled. They were interviewed and high vaginal swabs were taken for laboratory diagnosis by culture and wet mount microscopy. There were 601 participants in the age group of 15-45 years. Wet mount microscopy showed 68% sensitivity, 100% specificity, 100% positive (PPV) and 98% negative predictive values (NPV) (P=0.001, kappa=0.803) respectively against the gold standard culture. The area under the ROC curve was 0.840. Sensitivity of wet mount microscopy is low. However it has high validity and reliability as a specific diagnostic test for trichomoniasis. If it is to be used among women of reproductive age group in Western province, Sri Lanka, a culture method could be adopted as a second test to confirm the negative wet mount for symptomatic patients.

INTRODUCTION

Trichomoniasis is caused by the protozoan parasite *Trichomonas vaginalis*. It is transmitted via the sexual route. The associated correlations between trichomoniasis and other conditions such as HIV (Lehker *et al.*, 2000), obstetric and gynaecological complication (Viikki, 2000, Moodley *et al.*, 2002, Wendel, 2003, Soper, 2004) makes the proper diagnosis and treatment crucial.

The different approaches for the diagnosis are clinical and laboratory diagnosis. Clinical diagnosis is based on the complaints made by the patient and the findings in the physical examination. In trichomoniasis clinical signs and symptoms are non specific. No noticeable discomfort is experienced by more than 90% of infected

men and up to 50% of infected women (Cook *et al.*, 2009). Therefore laboratory tests are vital for definitive diagnosis prior to treatment.

The usual laboratory diagnostic techniques used in the diagnosis of trichomoniasis are wet mount microscopy, direct microscopy of stained preparations, culture, PCR, DNA probe technique, *Trichomonas* rapid dipstick test and latex agglutination test. Out of these, direct microscopy of the saline wet mount is the most commonly used method in clinical diagnostic services all over the world including Sri Lanka, as it is easy to perform, inexpensive and available in almost all the health care settings. In fact wet mount microscopy is the sole method of detecting trichomoniasis per se and the gold standard tests (culture and PCR) or other tests with higher validity are not yet available in the diagnostic services in Sri Lanka. Thus, it is important to validate the wet mount microscopy as it is the only available diagnostic test and has not been validated to date in Sri Lanka.

The objective of this study was to evaluate the validity and reliability of wet mount microscopy against gold standard *Trichomonas* culture among clinic based population of reproductive age group women in Western province, Sri Lanka.

MATERIALS AND METHODS

Women attending gynaecology clinics or well women clinics or sexually transmitted infection clinics in the government sector and health clinics owned by garment manufacturing factories in Western province, Sri Lanka were enrolled into this cross-sectional study. They belonged to the age group of 15-45 years. Stratified random sampling was used to select the respondents.

Exclusion criteria

Females with no history of sexual contact and virgins, refused vaginal or speculum examination, declined participation in the study, having menstruation and pregnant mothers were omitted. Women who had been on antibiotics during the preceding two weeks and at the time of data collection were also left out.

Data collection

Both symptomatic and asymptomatic women were included in the study since up to 50% of infected women can be asymptomatic in trichomoniasis. All the participants were interviewed for the collection of basic demographic data. Two fresh high vaginal swabs from the posterior fornix of the vagina using a self-retained bivalve cuscos speculum were obtained. The *Trichomonas* culture was inoculated using the first swab. The second swab was used for normal saline smear for microscopy as a bed side test.

Cystein-peptone-liver-maltose (CPLM) medium is an excellent and reliable medium for the isolation and culture of *Trichomonas vaginalis* (Lawrence *et al.*, 1987). *Trichomonas* modified CPLM medium base (HIMEDIA® Ref M 460) was used for this study. The preparation of culture media was done according to the manufacturer's instructions. Twelve millilitres of completed medium was dispensed into each sterile screw capped bottle and stored at 4°C. Every 3 weeks a new batch of medium was prepared discarding the remaining unused media. Prior to inoculation, bottles of culture media were allowed to reach room temperature. Inoculation of vaginal swab was done at the bed side. The swab was dipped into the culture medium and was rotated 10 times stirring the medium. The swab was squeezed against the side of the bottle wall before discarding it. Inoculated culture media were incubated at 37°C. They were examined daily up to the 5th day looking for motile trophozoites. Examination was done by taking a drop of culture medium from the bottom of the bottle and then preparing a wet mount to look under the microscope for motile trophozoites.

Wet mount for direct microscopy was prepared by mixing the fresh high vaginal swab from the posterior fornix in one or two drops of 0.9% normal saline solution on a clean grease free glass slide. A cover slip was placed on the smear and looked under the microscope for motile trophozoites.

Data analysis

The statistical software SPSS 16.0 was used for data entry and analysis. Sociodemographic data of the sample population were analysed and presented as frequency tables. Wet mount microscopy was validated against culture technique (gold standard laboratory test). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. ROC (Receiver-operating characteristic) curve was also drawn.

Ethical clearance

Ethical clearance was obtained from the ethics review committee of Colombo South Teaching Hospital and the ethics review committee of the Faculty of Medical sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

RESULTS

There were 601 participants in the age group of 15-45 years. The mean age of the participants was 31.7 years (SD = 8.8). The age categories of 15-25 years, 26-35 years and 36-45 years had almost equal representation in the sample; 31.4%, 32.1% and 36.4% respectively. The majority (68.1%) were married women while 27.5% were single and 4.5% were widows or legally separated. Overall, 51.6% were housewives.

All the participants have had vaginal sex at any time during the preceding year. There was one female with a bisexual relationship. Sexual practices of the population are given in Table 1. Out of females with multiple partners only 8.5% had trichomoniasis.

A past history of vaginal discharge was reported by 37.2% of participants. Overall 57.1% participants were symptomatic with vaginal discharge, itching, dysuria or lower abdominal pain either alone or in combination. Details of symptoms in the study population are given in Table 2. Past medical conditions relevant to trichomoniasis are given in Table 3.

There were 17 cases of wet mount positives for trichomoniasis. However, wet mount microscopy missed out 8 cases of

Table 1. Sexual practices in the study population

Sexual practices	Frequency (%)	(n=601)
Multiple partners		
Yes	71	(11.8)
No	530	(88.2)
Last Sexual encounter		
<3 months	416	(69.2)
>3 months	185	(30.8)
Use of condoms		
Yes	91	(15.1)
No	510	(84.9)

Table 2. Symptoms among participants in the study population

Symptoms	Frequency (%)	(n=601)
Vaginal discharge		
Yes	257	(42.8)
No	344	(57.2)
Itching		
Yes	127	(21.1)
No	474	(78.9)
Symptoms of urinary tract infections		
Yes	127	(21.1)
No	474	(78.9)
Back pain/ abdominal pain		
Yes	15	(2.5)
No	586	(97.5)

Table 3. Past medical conditions relevant to trichomoniasis

Past medical conditions	Frequency (%)	(n=601)
History of vaginal discharge		
Yes	223	(37.1)
No	378	(62.9)
History of treatment for vaginal discharge		
Yes	146	(65.5)
No	77	(34.5)
History of sexually transmitted infections		
Yes	172	(28.6)
No	429	(71.4)

culture positives. Of the total 25 cases of culture positives, 15 (60%) were asymptomatic. The evaluation of validity and reliability of wet mount microscopy against the gold standard culture showing sensitivity, specificity, PPV and NPV are given in Table 4. The area under the ROC curve was 0.840 as shown in Fig. 1.

DISCUSSION

Not much attention was given by the medical community to *Trichomonas* infection earlier due to it having fewer symptoms compared to the other STDs. However, with the expansion of knowledge linking it to HIV and gynaecological and obstetric adverse

Table 4. Validation of wet mount microscopy against *Trichomonas* culture

		Trichomonas culture (%)		
		Positive	Negative	Total
Wet mount microscopy (%)	Positive	17(100)	0(0)	17(2.8)
	Negative	8(1.4)	576(98.6)	584(97.2)
	Total	25(4.2)	576(95.8)	601(100)

P=0.001, kappa value=0.803

Sensitivity	68%
Specificity	100%
Positive predictive value (PPV)	100%
Negative predictive value (NPV)	98%

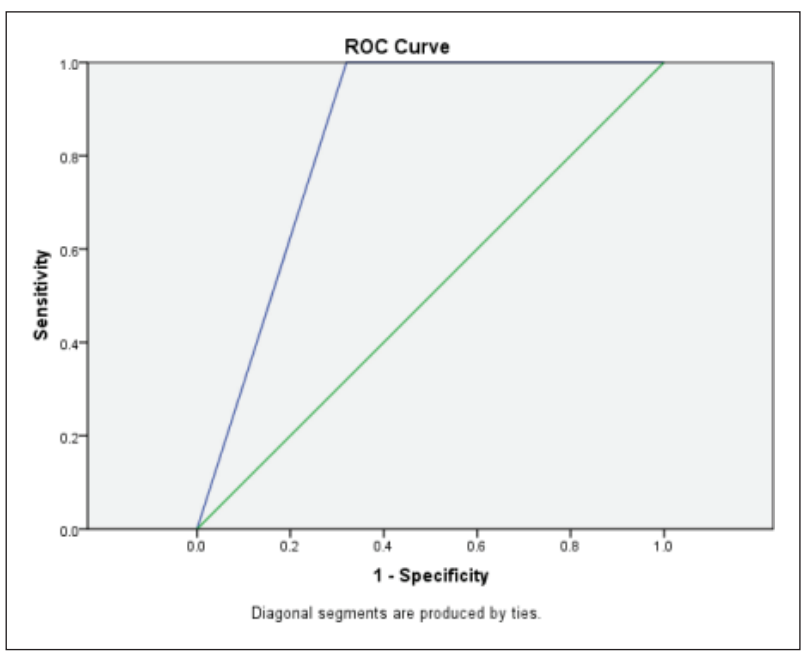

Figure 1. ROC curve
Area under the curve = 0.840.

outcomes, detection and treatment of trichomoniasis became essential.

The sensitivity of wet mount microscopy in this study was 68%. Sensitivity reported by a meta analysis ranges from 58-82% and our result falls within the stated range. Patil and collegues (2012) had reported 60% sensitivity when validated against PCR. Conversely there are other researchers who have reported sensitivity as high as 99.2% (Patel *et al.*, 2000, Huppert *et al.*, 2007, Pellati *et al.*, 2008, Nye *et al.*, 2009).

The specificity of wet mount microscopy in this study was 100%. Specificity of 97.7% to 100% have been reported by individual researchers (Patil *et al.*, 2012, Saleh *et al.*, 2014) whilst a meta-analysis has published a specificity of 99.8% (Wiese *et al.*, 2000) for wet mount.

Sensitivity and specificity of a test change with disease prevalence. Spectrum of the selected patient population affects the prevalence of studies and thereby sensitivity and specificity (Leeflang *et al.*, 2013). For example, a study (Huppert *et al.*, 2007) (n=594) conducted among women attending a sexually transmitted diseases clinic (hospital based) had 50.8% sensitivity while a study(Nye *et al.*, 2009) (n=330) carried out to screen sexually active adolescent women attending a teen health centre (community based) had a sensitivity of 54.6% for wet mount microscopy. Thus studies from different settings and populations do not yield the same sensitivity results even for the same diagnostic test.

The area under the ROC curve was high indicating wet mount microscopy has a higher ability to discriminate between those individuals with the disease and without the disease.

Wet mount microscopy is a simple procedure, giving fast results, needing only a microscope and a trained personnel. For these reasons it is the test of choice not only in resource poor settings but worldwide. However, trichomoniasis is significantly under diagnosed with the current diagnostic standard of wet mount microscopy (Soper, 2004). The sensitivity of wet mount microscopy is highly dependent on the skills and experience of the microscopist.

Sensitivity is also affected by the delay in transporting or processing as organisms often die during transfer. It contributes to lower sensitivity. Specimens should be examined within 10 to 20 minutes of collection for better results as when delayed the organism loses its motility. Kingston *et al.*, 2003) had concluded that one fifth of wet mount preparations initially positive for *T. vaginalis* become apparently negative within 10 minutes of the immediate initial reading. Schwebke and collegues (2004) documented survival of *T. vaginalis* up to 24 hours in Amies gel agar medium. Lower sensitivity may occur if the number of parasites present is low or if there is excessive inflammatory response concealing the parasite. On rare occasions ciliated bodies from epithelial cells of the genital tract may be mistakenly identified as some parasite (WHO, 1991). Temperature above 40ºC or drying in the sun can kill the parasite too.

Specificity of wet mount remains high as it involves direct visualization of the parasite. *T. vaginalis* can be easily identified in the wet mount by its pear shape five flagella and its nervous twitching, jerky or jumpy movements. It may be seen beating its flagella at rest. The organisms are about the size of a white blood cell. Since *T. vaginalis* is the only species of *Trichomonas* that inhabits the urogenital system there is no need to study the morphological features or to differentiate it from *Pentatrichomonas hominis* which lives in the intestine (WHO, 1991).

In conclusion, the sensitivity of wet mount microscopy is low, thus it is unsuitable for trichomoniasis screening among women of reproductive age group in Western province, Sri Lanka. However it has high validity and reliability as a specific diagnostic test for trichomoniasis. Since the negative wet mount does not rule out the infection with *T. vaginalis*, it is mandatory to perform a gold standard laboratory method as a second test for those with symptoms.

If wet mount microscopy is to remain as the sole diagnostic test for trichomoniasis, an alternative test with higher sensitivity should be made available to confirm the wet mount negatives. *Trichomonas* culture, OSOM *Trichomonas* rapid test and latex

agglutination test are possible options with high sensitivity and specificity.

Acknowledgment. We wish to thank all the participants and University of Sri Jayewardenepura, Nugegoda, Sri Lanka for finding the project. (University grant ASP/06/RE/MED/2005/09)

REFERENCES

Cook, G. & Zumla, A. (2009). Manson's tropical diseases, Saunders an imprint of Elsevier Limited.

Huppert, J.S., Mortensen, J.E., Reed, J.L., Kahn, J.A., Rich, K.D., Miller, W.C. & Hobbs, M.M. (2007). Rapid antigen testing compares favorably with transcription-mediated amplification assay for the detection of trichomonas vaginalis in young women. *Clinical Infectious Diseases* **45**(2):194-198.

Kingston, M.A., Bansal, D. & Carlin, E.M. (2003). 'shelf life' of trichomonas vaginalis. *International Journal of STD & AIDS* **14**(1): 28-29.

Lawrence, R.A. & Thomas, C.O. (1987). Parasites: A guide to laboratory procedures and identification, American Society of Clinical Pathologists Press.

Leeflang, M.M., Rutjes, A.W., Reitsma, J.B., Hooft, L. & Bossuyt, P.M. (2013). Variation of a test's sensitivity and specificity with disease prevalence. *Canadian Medical Association Journal* **185**(11): E537-544.

Lehker, M.W. & Alderete, J.F. (2000). Biology of trichomonosis. *Current Opinion in Infectious Diseases* **13**: 37-45.

Moodley, P., Wilkinson, D., Connolly, C., Moodley, J. & Sturm, A.W. (2002). *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clinical Infectious Diseases* **34**(4): 519-522.

Nye, M.B., Schwebke, J.R. & Body, B.A. (2009). Comparison of aptima trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. *American Journal of Obstetrics & Gynecology* **200**(2): 188 e181-187.

Patel, S.R., Wiese, W., Patel, S.C., Ohl, C., Byrd, J.C. & Estrada, C.A. (2000). Systematic review of diagnostic tests for vaginal trichomoniasis. *Infectious Diseases in Obstetrics and Gynecology* **8**(5-6): 248-257.

Patil, M.J., Nagamoti, J.M. & Metgud, S.C. (2012). Diagnosis of trichomonas vaginalis from vaginal specimens by wet mount microscopy, in pouch tv culture system, and pcr. *Journal of Global Infectious Diseases* **4**(1): 22-25.

Pellati, D., Mylonakis, I., Bertoloni, G., Fiore, C., Andrisani, A., Ambrosini, G. & Armanini, D. (2008). Genital tract infections and infertility. *European Journal of Obstetrics Gynecology and Reproductive Biology* **140**(1): 3-11.

Saleh, A., Abdalla, H., Satti, A., Babiker, S., Gasim, G. & Adam, I. (2014). Diagnosis of trichomonous vaginalis by microscopy, latex agglutination, diamond's media, and pcr in symptomatic women, khartoum, sudan. *Diagnostic Pathology* **9**(1): 49.

Schwebke, J.R. & Burgess, D. (2004). Trichomoniasis. *Clinical Microbiology Reviews* **17**(4): 794-803.

Soper, D. (2004). Trichomoniasis: Under control or undercontrolled? *American Journal of Obstetrics & Gynecology* **190**(1): 281-290.

Viikki, M. (2000). Gynaecological infections as risk determinants of subsequent cervical neoplasia. *Acta Oncologica* **39**(1): 71-75.

Wendel, K. (2003). Trichomoniasis: What's new? *Current Infectious Disease Reports* **5**(2): 129-134.

WHO (1991). Basic laboratory methods in medical parasitology, Geneva, Switzerland, World Health Organization.

Wiese, W., Patel, S.R., Patel, S.C., Ohl, C.A. & Estrada, C.A. (2000). A meta-analysis of the papanicolaou smear and wet mount for the diagnosis of vaginal trichomoniasis. *American Journal of Medicine* **108**(4): 301-308.