

## Original Article

# Evaluation of an Immunochromatographic Strip (Xenostrip – Tv) Test for Diagnosis of Vaginal Trichomoniasis Compared with Wet Mount and PCR Assay

S Maraghi<sup>1</sup>, A Khosravi<sup>2</sup>, T Kardooni<sup>1</sup>, T Razi<sup>3</sup>, MH Feiz-Haddad<sup>4</sup>

<sup>1</sup> Dept. of Parasitology and Mycology, Thalassemia and Haemoglobinopathy Research Centre, Tropical and Infectious Diseases Research Centre, Jondi-Shapour University of Medical Sciences, Ahwaz, Iran

<sup>2</sup> Microbiology Dept. Tropical and Infectious Diseases Research Centre, Jondi-Shapour University of Medical Sciences, Ahwaz, Iran

<sup>3</sup> Obstetric and Gynaecolog Ward, Medical School, Jondi- Shapour University of Medical Sciences, Ahwaz, Iran

<sup>4</sup> Dept. of Parasitology and Mycology, School of Medicine, Jondi-Shapour University of Medical Sciences, Ahwaz, Iran

(Received 29 Dec 2007; Accepted 18 Aug 2008)

## Abstract

**Background:** Trichomoniasis, caused by *Trichomonas vaginalis*, is one of the most common sexually transmitted infections in the world. Diagnosis of *T. vaginalis* is performed by different methods, including wet mount, culture, serological methods and PCR, which required laboratory equipments and expert laboratory personnel. The aim of this study was evaluation of immunochromatographic strip test (Xenostrip-Tv) for diagnosis of vaginal trichomoniasis compared with wet mount and PCR assay.

**Methods:** In this prospective study vaginal swabs were obtained from 100 women with genital complaints demanding a speculum examination, referred to Imam Khomeini and Amir Kabir hospitals in Ahwaz, Khuzestan Province. Samples were first examined by wet mount and Xenostrip-Tv. PCR assay was performed in the next step using TVK3 and TVK7 primers initially. The positive samples were then confirmed by the second PCR assay using TVA5-1 and TVA6 primers.

**Results:** PCR with TVA5-1 and TVA6 primers was determined as gold standard. The wet mount as well as Xenostrip-Tv sensitivity and specificity were 73.3% and 100%, respectively in comparison with gold standard. The sensitivity and specificity of PCR with primers TVK3 and TVK7 were also determined as 100% and 96.6%, respectively. The infection rates were 14% for wet mount and Xenostrip-Tv, 21% for PCR with primers TVK3 plus TVK7 and 19% with the gold standard PCR using TVA5-1 and TVA6 primers.

**Conclusion:** Xenostrip- Tv could be used for diagnosis of vaginal trichomoniasis in regions with no laboratory diagnostic facilities.

**Keywords:** *Trichomonas vaginalis*, *Xenostrip-Tv*, *Wet mount*, *PCR*

## Introduction

Vaginal trichomoniasis is one of the most common sexually transmitted diseases in the world, accounting for approximately 180 million infections annually (1). In the United

States, it is responsible for an estimated 5 million new infections annually (2). It is recognized as a minor cause of urethritis in men (3). In addition, *T. vaginalis* infection has been shown to be associated with an increase in risk of acquisition and transmission of the

human immunodeficiency virus (4-5). An association of pelvic inflammatory disease, tubule infertility, and cervical cancer with previous episodes of trichomoniasis has been reported but may be explained by its association with other STDs (6-8). Complications of trichomonal vaginitis that have been reported include premature rupture of membranes, premature labor, low birth weight, and post-abortion or post-hysterectomy infection (9-12).

The most common method of *T. vaginalis* detection is wet-mount microscopy. Although this technique is inexpensive and provides immediate results, it is a subjective test that requires clinical experience and access to a microscope. Even in the hands of trained observers, the wet mount is only 36 to 75% sensitive compared to culture (13). Culture of trichomonads from vaginal swab specimens and urine (14, 15) remains the "gold standard" against which the performance of other diagnostic methods is measured. Unfortunately, culture is time-consuming, with results usually being available only 48 to 72 h after inoculation of the culture medium. In addition, it is impractical in many settings since specialized laboratory equipments such as incubator and microscope is required. Recently, a number of PCR assays (16, 17) have been developed for the detection of *T. vaginalis* and these assays have generally proved more sensitive than culture; however, PCR also requires a dedicated laboratory, sophisticated equipments, and specially trained technicians

In this study, an immunochromatographic strip test (Xenostrip-Tv) was evaluated for detection of *T. vaginalis* by comparing with wet mount and PCR assay using vaginal swab specimens.

## **Materials and Methods**

In this prospective study the samples were collected from the vaginal discharge of 100 women at the age of 20  $\geq$  49 years with clinical symptoms referred to the clinics of Obstetric

and Gynecology wards in Imam Khomeini and Amir-kabir hospitals Ahwaz, Khuzestan Province, Iran from May 2005 to March 2006.

Four vaginal swabs were obtained from each patient. The first swab was placed in 0.5 ml of 0.9% saline and wet mount was prepared on slide and examined microscopically, the second swab was placed in the test tube with 0.5 ml of 0.9% buffer test (OSOM) available in the kit. The swab was squeezed for one minute and then was thrown away. The strip (Genzyme diagnostic, UK) entered the test tube containing the vaginal discharge in buffer and after 10 minutes, the result was read. If *T. vaginalis* was present in the specimen, the blue line would appear on the strip. The control line (negative) would appear as red (Fig. 1). The third and fourth swabs were entered in tubes containing 0.9% sterile normal saline, squeezed, and threw away. The samples were kept in -70°C until use for PCR.

### **DNA extraction**

After thawing, the content of each tube was placed into 1.5 ml microtube and centrifuged (5000xg) for 5 minutes and then the precipitate was washed with PBS. After 2 times washing, 200  $\mu$ l of digestive buffer (Tris-Hcl- 0.5M, EDTA- 0.1M), and Tween 20 were added to the precipitate followed by adding immediately 9  $\mu$ l proteinase K to each microtube (concentration equal to 50 mg/ml). Microtubes were vortexed for some seconds and then for one night they were kept in incubator at 37° C or for 2 hours at 56° C until proteinase K digest protein available in the sample. After passing this period, 200  $\mu$ l soluble of phenylchloroform isoamyl alcohol was added and then microtubes were vortexed for one min and centrifuged (1000g) for 10 minutes. After centrifugation, a soluble of two phases produced, including the upper phase as soluble DNA and the lower one as phenyl as well as fat. The upper phase was separated accurately and slowly by sampler and poured in 1.5 ml microtube and the microtubes containing phenyl and fat were thrown away.

To each microtube, 400 µl ethanol about double volume of liquid was added, and then the microtubes were kept in -20° C for 45-60 minutes. The microtubes were removed and centrifuged for 10 minutes (10000xg). The upper liquid was thrown away by sampler and DNA precipitate and a very little of ethanol was remained at the bottom of microtubes which this sediment became dry by keeping at room temperature for one night or for some hours. On dried sediment, 50µl sterile deionized distilled water was added and kept in 37° C for 30-60 minutes until the dissolving of sediment took place slowly, but during the utilization, microtubes must be vortexed for some seconds (18).

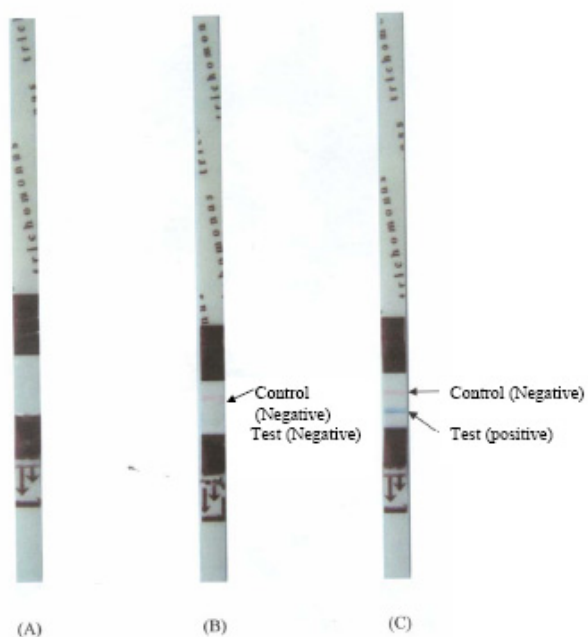
The PCR was conducted with two sets of primers. First, the samples were amplified with two pairs of primers, TVK<sub>3</sub> (5'-AT TGT CGA ACA TTG GTC TTA CCC TC-3') and TVK<sub>7</sub> (5'-TCT GTG CCG TCT TCA AGT ATG C-3') (19), and in the next step only samples which recorded with these sets of primers were accepted. The reaction volume (25µl) composed of 50 m mol KCl, 10 m mol Tris- HCl (pH 8.3), 1.5 m mol Mg Cl<sub>2</sub>, 0.2 m mol of each deoxynucleotide triphosphate, 0.5 m mol of each set of primers, TV A5- 1 (5\_ AT GTTCTA TCT TTT CAT TGT-3) and TV A6 primers(5- GAT CAC CAC CTT AGT TTA C3)(20), 1.25 units of Taq polymerase, 9 µl of double distilled water and 10 µl of DNA template. A drop of mineral oil was added to each microtube and all microtubes were placed in a Themocycler (Techgene, UK). The reaction conditions were programmed for TV K3 and TV K7 primers as follows: An initial denaturation step at 90° C for 5 min followed by 45 cycles of DNA denaturation at 90° C for 1min,

primer annealing at 60° C for 30 s and primer extension at 72° C for 1min and a final extension at 72° C for 7 min. Reaction condition for PCR using TVA5-1 and TVA6 primers were slightly different as below: An initial denaturation step at 95° C for 5 min followed by 45 cycles of DNA denaturation at 94° C for 3 min, primer annealing at 46° C for 1min and primer extension at 72° C for 1 min, and a final extension at 72° C for 7 min. The PCR products were loaded on 2% (w/v) agarose gel with 0.5 mg/ml of ethidium bromide and were analyzed by gel electrophoresis. *T. vaginalis* in the presence of TV K3 and TV K7 primers yielded a 261 base pair PCR product and with TV A5-1 and TV A6 primers was able to produce a 98 base pair PCR product (Fig. 2).

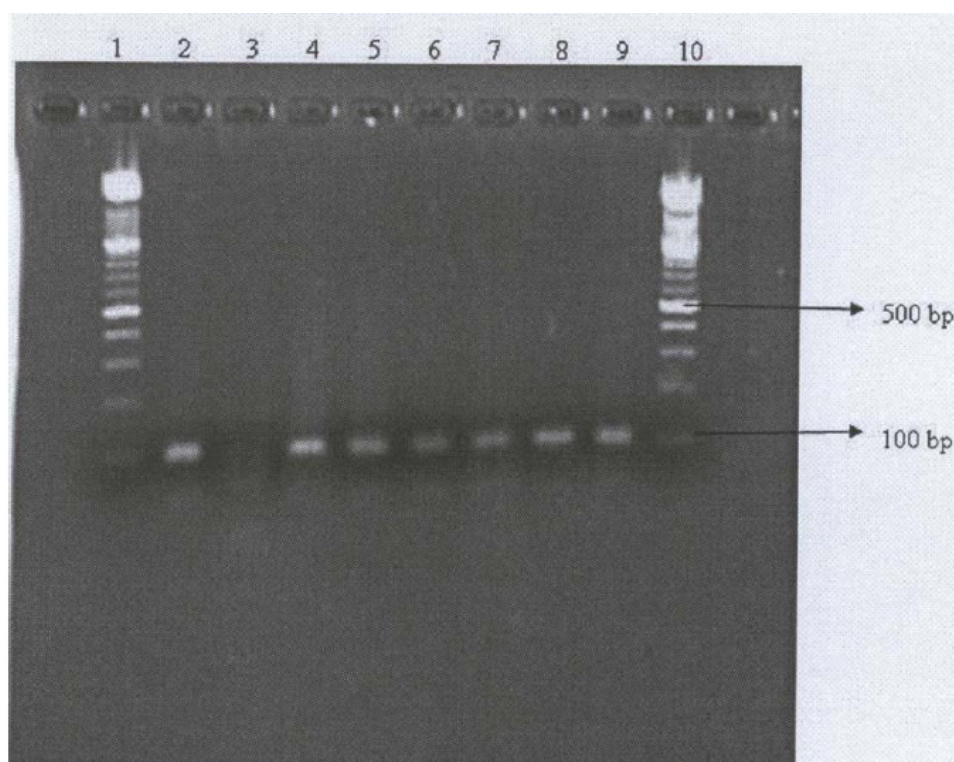
## Results

Fourteen out of 100 individuals were diagnosed to be infected with *T. vaginalis* by the methods of wet mount and Xxenostrip- Tv. The age of patients were from 20 ≥49 years. Ninety five percent of the cases were married. The pH of vagina in 63% of patients was lower than 6. Totally 14 cases with direct method and Xenostrip-Tv and 21 cases with PCR (primer TVK<sub>3</sub> and TVK<sub>7</sub>) were positive for vaginal trichomoniasis.

The infection rate of *T. vaginalis* was 19% with golden standard PCR method using primers, TVA5-1 and TVA6. The sensitivity and specificity of wet mount and Xenostrip- Tv methods in comparison with golden standard were 73.3% and 100%, respectively for both tests and for PCR with primer sets of TVK<sub>3</sub> and TVK<sub>7</sub> were 100% and 96.6%, in that order.



**Fig. 1:** Xenostrip – Tv. Unused strip (A). Negative test (B) and positive test (C)



**Fig. 2:** Electrophoresis of PCR product with TVA6 and TVA 5-1 primers on 2% agarose gel. Lane 1 and 10 with 100 bp (Markers). Lane 2 (positive control), Lane 3 (Negative control). Lanes 4, 5, 6, 7, 8 and 9 are patients' samples

## **Discussion**

The most common method for diagnosis of *T. vaginalis* is wet mount and watching motile trichomonad under microscope. Though this method is cheap and easy to practice, but its sensitivity compared to culture method is low and it is variable between 36 to 75% (20). In some studies, its sensitivity in compare to PCR method was reported to be 34.5 to 58.5% (17, 21- 23).

Culture is usually estimated to be the 'gold standard' for diagnosis. But this method is not available in routine and only for research work is applicable. Because of its high cost and incubation time (2-7 days), infected untreated patients can transmit the disease during this period of time (24, 25). Though the sensitivity of Xenostrip-Tv in this study was equal to wet mount, but it is more preferable than wet mount.

As wet mount method needs microscope and laboratory profile, and physicians must sent the samples to the laboratory, and on the other hand it is not always possible to do and get the laboratory results, so physicians do not send the samples to the laboratory and only according to clinical diagnosis treat the patients. Due to some reasons clinical diagnosis can not be the basis for disease detection. First, due to a similarity of clinical signs to other sexual diseases second, strawberry like cervix which is specific sign of the disease is only seen in 2% of *T. vaginalis* infected patients, and third, foam like discharges are observed only in 12% of patients. If the only classic form of the disease used for trichomoniasis diagnosis, 88% of infected women cannot be diagnosed and 29% are misdiagnosed.

These data show that clinical symptoms are not valid parameters for diagnosing the disease and laboratory researchers believe that correct diagnosis for trichomoniasis is necessary, because particular treatment is done and control of infection development becomes easy (26,27).

The Xenostrip- Tv test is a qualitative assay that detects *T. vaginalis*-specific antigen by color immunochromatographic (dipstick) technology with mouse antibodies bound to a nitrocellulose membrane .and is more applicable than wet mount preparation and everybody can use it in different clinics. Its sensitivity was equal to wet mount method in the present study while in Pillay *et al.* study (1) the sensitivity and specificity of Xenostrip–Tv were 66.6% and 100%, respectively compared to those of expanded gold standard and the sensitivity and specificity of wet mount compared to those of gold standard were 48.1% and 100%. In another study, the sensitivity and specificity of OSOM vaginal specimens were 83.3% and 98.8%, respectively while wet mount had a sensitivity and specificity of 71.4% and 100%, respectively compared to CRS (28).

Although in the present study, the sensitivity of Xenostrip method in comparison with PCR was low but detection of infection in vaginal discharge samples occur faster and in a period of minutes or less without need to laboratory equipments.

As previously discussed, the sensitivity of PCR was higher than Xenostrip-Tv and direct method in the present study, which was in agreement with previous studies (29, 30) but due to problems in application of PCR for routine use, the technique is recommended only for research works.

It is concluded that Xenostrip-Tv method is more applicable than PCR, culture and even wet mount in regions with no laboratory facilities and could be done by the physicians even in rural areas.

## **Acknowledgments**

We would like to appreciate the financial support of Vice Chancellor of Jondi Shapour University Research Affairs and express our thanks to the staff of Imam Khomeini and Amir

kabir clinics and Genzyme Diagnostic Company. We are also grateful to Miss A. Sarkaki and M. Esfahani for their cooperation. The authors declare that they have no conflict of interests.

## References

1. Pillay A, Lewis J, Ballard RC. Evaluation of Xenostrip-Tv, a rapid diagnostic test for *Trichomonas vaginalis* infection. *J Clin Microbiol.* 2004; 42, 3853-3859.
2. Cates WJr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. *Sex Transm.* 1999;26:52-57.
3. Schwebke JR, Hook III EW. High rates of *Trichomonas vaginalis* among men attending a sexually transmitted diseases clinic: implications for screening and urethritis management. *J Infect Dis.* 2003;188:465-468.
4. Sorvillo F, Kerndt P. *Trichomonas vaginalis* and amplification of HIV-1 transmission. *Lancet.* 1998;351:213-214.
5. Guenther PC, Secor WE, Dezzutti CS. *Trichomonas vaginalis*-induced epithelial monolayer disruption and an immunodeficiency virus Type1 (HIV-1) replication: implications for the sexual transmission of HIV-1. *Infect Immun.* 2005;73:4155-4160.
6. Okonofua FE, Ako-Nai KA, Dighitoghi MD. Lower genital tract infection in infertile Nigerian women compared with controls. *Genitourin.* 1995;71:163-168.
7. Paisarntantiwong R, Brockmann S, Clarke L. The relationship of vaginal trichomoniasis and pelvic inflammatory disease among women colonized with *Chlamydia trachomatis*. *Sex Transm Dis.* 1995;22:344-347.
8. Zhang ZF, Graham S, Yu SZ. *Trichomonas vaginalis* and cervical cancer: a prospective study in China. *Ann Epidemiol.* 1995;5:325-332.
9. Hardy PH, Hardy JB, Nell EE, Graham DA, Spence M. R. & Rosenbaum R.C. Prevalence of six sexually transmitted disease agents among pregnant inner-city adolescents and pregnancy outcome. *Lancet ii.* 1984;333-337.
10. Krieger JN, Tam MR, Stevens CE, Nielsen IO, Hale J, Kiviat NB, Holmes KK. Diagnosis of trichomoniasis. *JAMA.* 1998;259:1223-1227.
11. Sobel JD. Vaginitis. *N Engl J Med.* 1996;337:1896-1903.
12. Soper DE, Bump RC, Hurt WG. Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulites after abdominal hysterectomy. *Am J Obstet Gynecol,* 1990;163:1016-1021.
13. Wiese W, Patel SR, Patel SC, Ohl CA, Estrada CAA. Meta-analysis of the Papanicolaou smear and wet mount for the diagnosis of vaginal trichomoniasis. *Am J Med,* 2000;108:301-308.
14. Borchardt KA, Smith RF. An evaluation of an Inpouch Tv culture method for diagnosis *Trichomonas vaginalis* infection. *Genitourin. Med,* 1991;67:149-152.
15. Schwebke, JR, Lawing LF. Improved detection by DNA amplification of *Trichomonas vaginalis* in males. *J Clin Microbiol.* 2002;40:3681-3683.
16. Lawing LF, Hedges SR, Schwebke JR, Detection of Trichomoniasis in urine specimens from women by culture and PCR. *J Clin Microbiol.* 2000; 38:3585-8.
17. Madico G, Quinn TC, Rompalo K, Mckee T, Gaydos CA. Diagnosis of *Trichomonas vaginalis* by PCR using vaginal swab samples. *J Clin Microbiol.* 1998; 36:3205-3210.
18. Mc Pherson MG, Moller SG. PCR: The basic from background to bench. First

- edition. Bios publication. UK, 2000; pp.1-83.
19. Kengne P, Veas F, Vidal N, Rey JL, Cuny G. *Trichomonas vaginalis*: repeated DNA target for highly Sensitive and specific polymerase chain reaction diagnosis. *Cell Mol Biol.* 1994;40:819-831.
  20. Riley DE, Roberts MC, Takayama T, Krieger JN. Development of a polymerase chain reaction-based diagnosis of *Trichomonas vaginalis*. *J Cini Microbiol.* 1992;30:465-472.
  21. Krieger JN, Tam MR, Stevens CE, Nielsen IO, Hale J, Kiviat NB, Holmes KK. Diagnosis of trichomoniasis. *JAMA.* 1998;259:1223-1227.
  22. Mahmoud MS, Abdel-Aziz SS, El-Sherif, EA, Swidan KH. Diagnosis of symptomatic and asymptomatic *Trichomonas vaginalis* infection by applying one tube nested PCR to vaginal discharge. *J Egypt Soc Parasitol.* 1999;29:1031-1046.
  23. Rampalo AM, Gaydos CA, Shah N, Tennant M, Crotchfelt KA, Madico G, *et al.* Evaluation of use of a single intravaginal swab to detect multiple sexually transmitted infections in active-duty military women. *Clin Infect Dis.* 2001;33:1455-1461.
  24. Wendel KA, Erbeding EJ, Gaydos CA, Rampalo AM. *Trichomonas vaginalis* polymerase chain reaction compared with standard and therapeutic protocols for detection and treatment of vaginal trichomoniasis. *Clin Infect Dis.* 2002;35: 576-580.
  25. Draper D, Parker R, Patterson E, Jones W, Beuez M, French J, *et al.*, Detection of *Trichomonas vaginalis* in pregnant women with the Inpouch Tv system. *J Clin Microbiol,* 1993;31:1016-1018.
  26. Schwebke JR, Burgess D. Trichomoniasis. *Clin Microbiol Rev.* 2004;17:794-803.
  27. Petrin D, Delgaty K, Bhatt R, Garber E. Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clin Microbiol Rev.* 1998;11:300-317.
  28. Huppert TS, Batteiger BE, Brasilins P, Feldman JA, Hobbs MM, Sankey H.Z, *et al.* Use of an immunochromatographic assay for rapid detection of *Trichomonas vaginalis* in vaginal specimens. *J Clin Microbiol,* 2005;43:684-687.
  29. Ryu J, Chung H, Min D, Cho YH, Ro YS, Kim SR. Diagnosis of trichomoniasis by polymerase chain reaction. *Yonsei Med J.* 1999;40:56-60.
  30. Schee C, Belkun A, Zwijgers Lk, Brugge E, O'Neill, EL, Luijendijk, A, *et al.* Improved diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swabs and urine specimens compared to diagnosis by wet mount microscopy, culture and fluorescent staining. *J Clin Microbiol.* 1999;37: 4127-4130.