JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2004, p. 3853–3856 Vol. 42, No. 8
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.8.3853–3856.2004

Evaluation of Xenostrip-Tv, a Rapid Diagnostic Test for *Trichomonas vaginalis* Infection

A. Pillay,* J. Lewis, and R. C. Ballard

Sexually Transmitted Infections Branch, Division of Sexually Transmitted Diseases Prevention, NCHSTP, Centers for Disease Control and Prevention, Atlanta, Georgia

Received 27 January 2004/Returned for modification 30 March 2004/Accepted 21 April 2004

An immunochromatographic strip test, Xenostrip-Tv, was compared to wet mount and PCR for the diagnosis of *Trichomonas vaginalis* infection in women. Of 428 specimens tested, 54 (12.6%) were positive by an "expanded gold standard," defined as either a positive wet mount and PCR test with primers TVK3 and TVK7 and/or a positive PCR test confirmed by a second PCR assay with primers TVA5-1 and TVA6; 26 (6%) were positive by wet mount, and 36 (8.4%) were positive by Xenostrip-Tv test. Since the Xenostrip-Tv test is rapid and easy to perform and proved to be more sensitive than wet mount, it should be considered as an alternative to wet mount for point-of-care diagnosis of trichomoniasis, especially in settings where microscopy is impractical.

Trichomoniasis, caused by *Trichomonas vaginalis*, is one of the most common sexually transmitted infections in the world, accounting for approximately 180 million infections annually (19). In the United States, it is responsible for an estimated 5 million new infections annually (2) and infection during pregnancy has been associated with preterm labor and low birth weight (4). It is also recognized as a minor cause of urethritis in men (16). 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 (3, 7).

Culture of trichomonads from vaginal swab specimens and urine (1, 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. Also, it is impractical in many settings since specialized laboratory equipment such as incubators and microscopes is required. Recently, a number of PCR assays (8, 9) 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 equipment, and specially trained laboratorians. Microscopic examination of a wet preparation of vaginal fluid remains the most widely used rapid diagnostic technique for the laboratory detection of *T. vaginalis* in the United States. It is less sensitive than both culture and PCR but has the advantage of being a rapid, cost-effective method that is easy to perform and can provide a point-of-care diagnosis.

In this study, we evaluated an immunochromatographic strip test, Xenostrip-Tv (Xenotope Diagnostics Inc., San Antonio, Tex.), for the detection of *T. vaginalis* by comparing it to wet mount and a PCR assay using vaginal swab specimens.

(Part of the data in this study was presented at the 18th Congress on Sexually Transmitted Infections IUSTI-Europe in Vienna, Austria, September 2002.)

Vaginal swabs were obtained from 428 consecutive women presenting with genital tract complaints necessitating a speculum examination at three adult health centers in the Atlanta, Ga., metropolitan area. Swabs were placed into sterile 10-ml screw-cap plastic tubes containing 0.5 ml of 0.9% saline and transported to the on-site laboratory for processing. Each swab was vigorously rotated in the saline and pressed against the side of the tube to express as much fluid as possible prior to removal. A wet preparation was performed with the expressed fluid, and slides were read within 20 min of specimen collection. Briefly, a drop of the expressed fluid was placed on a glass slide and examined under a microscope. The presence of motile trichomonads was considered a positive test. Thereafter, all patient identifiers were removed from the specimen tubes before being transported on ice to the Centers for Disease Control and Prevention for testing. Specimens were aliquoted and stored at 4°C, and the Xenostrip-Tv test was performed within 24 h and PCR was performed within 2 months. This study was determined to be exempt from institutional review board approval.

The Xenostrip-Tv test (Xenotope Diagnostics) is a qualitative assay that detects *T. vaginalis*-specific antigen by color immunochromatographic "dipstick" technology with mouse antibodies bound to a nitrocellulose membrane. If *T. vaginalis* is present in a specimen, a specific trichomonad antigen forms a complex with the primary murine anti-trichomonas antibody conjugated to red particles, and the secondary anti-mouse capture antibodies will then bind to the antigen complex. A red test line indicates a positive result. A second capture line, comprising rabbit anti-mouse antibodies, is incorporated into each test strip to capture any excess primary anti-trichomonas antibodies. This also forms a red test line and serves as a positive control, monitoring antibody stability and performance. A red control line but no red test line is a presumptive negative result, and if no red control line appears or background color makes the reading impossible, then the result is invalid. Ten drops of sample buffer (Xenotope Diagnostics) was added to each specimen tube, the absorbent end of a

* Corresponding author. Mailing address: Sexually Transmitted Infections Branch, Division of Sexually Transmitted Diseases Prevention, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS-G39, Atlanta, GA 30333. Phone: (404) 639-2140. Fax: (404) 639-3976. E-mail: Apillay@cdc.gov.

TABLE 1. Comparison of diagnostic tests of 428 specimens for *T. vaginalis*[a]

Assay or test	No. of tests				% Sensitivity (95% CI)	% Specificity (95% CI)	% Predictive value	
	True positive	False positive	False negative	True negative			Positive	Negative
Wet mount	26	0	28	374	48.2 (34.4–62.2)	100 (99–100)	100	93
Xenostrip-TV	36	0	18	374	66.7 (52.6–78.9)	100 (99–100)	100	95.4
PCR	54	3	0	371	100 (93.4–100)	99.2 (97.7–99.8)	94.7	100

[a] The gold standard used was a positive wet mount confirmed by PCR with primers TVK3 and TVK7 and/or a positive PCR test confirmed by a second PCR assay with primers TVA5-1 and TVA6 (n = 54). CI, confidence interval.

Xenostrip test strip was placed in the tube, and the results were read at 10 min. The results were interpreted as follows: a red test line indicated a positive result, a red control line but no red test line was a presumptive negative result, and if no red control line appeared or if the test could not be interpreted because of high background color then the result was determined to be invalid. All Xenostrip-Tv test kits were provided by Xenotope Diagnostics. The Xenostrip-Tv test has been licensed to Genzyme Diagnostics, a division of Genzyme General, Cambridge, Mass.

DNA was extracted from specimens with the QIAamp DNA mini kit (QIAGEN, Valencia, Calif.) in accordance with the manufacturer's instructions. A PCR assay with primers TVK3 (AT TGT CGA ACA TTG GTC TTA CCC TC) and TVK7 (TCT GTG CCG TCT TCA AGT ATG C) was used to amplify a 261-bp sequence of a *T. vaginalis*-specific repeat DNA fragment (6). Briefly, the PCR was performed in a reaction volume of 50 μl with the following components: 1 μl of deoxynucleoside triphosphates (0.2 mM each dATP, dCTP, dGTP, and dTTP; Roche Diagnostics, Branchburg, N.J.), 0.75 μl of dUTP (0.3 mM; PE Applied Biosystems, Foster City, Calif.), 6 μl of $MgCl_2$ (1.5 mM; PE Applied Biosystems), 0.2 μM each primer, 1 U of uracil DNA glycosylase (Roche Diagnostics), 2.5 U of AmpliTaq Gold polymerase (PE Applied Biosystems), 10 μl of 10× PCR buffer (PE Applied Biosystems), and 20 μl of template DNA. The forward primer (TVK3) was labeled at the 5′ end with a fluorescein phosphoramidite label, which enabled amplicon size determination on the ABI 310 Genetic Analyzer (PE Applied Biosystems). PCR amplification consisted of two hold cycles: 20°C for 10 min and 95°C for 5 min, followed by 45 cycles of 90°C for 1 min, 60°C for 30 s, and 72°C for 1 min. The final extension consisted of one cycle of 72°C for 7 min. Specimens that were found to be positive by PCR with primers TVK3 and TVK7 but negative by either a Xenostrip-Tv test or a wet preparation were retested with a confirmatory PCR assay. The confirmatory assay used primers TVA5-1 (AT GTT CTA TCT TTT CAT TGT) and TVA6 (GAT CAC CAC CTT AGT TTA CA) to amplify a 98-bp fragment of a *T. vaginalis*-specific sequence (9). PCR was performed in a reaction volume of 50 μl with the following components: 1 μl of deoxynucleoside triphosphates (0.2 mM each dATP, dCTP, dGTP, and dTTP; Roche Diagnostics), 0.2 μM each primer, 1.75 U of Expand *Taq* polymerase (Roche Diagnostics), 10 μl of 10× PCR buffer containing $MgCl_2$ (Roche Diagnostics), and 20 μl of template DNA. The forward primer (TVA5-1) was labeled with fluorescein phosphoramidite so that amplicon size could be determined on the ABI 310 Genetic Analyzer (PE Applied Biosystems). PCR amplification consisted of an initial hold cycle of 95°C for 5 min, followed by 45 cycles of 94°C for 30 s, 47°C for 1 min, and 72°C for 1 min. The final extension consisted of one cycle of 72°C for 7 min. All PCR amplifications were performed in a GeneAmp PCR system 9700 (PE Applied Biosystems). PCR amplicon sizes were determined with an ABI 310 Genetic Analyzer as previously described (12). Briefly, a peak on the electropherogram corresponding to either a 261-bp or a 98-bp fragment was considered a positive PCR result. When no visible peak was present this specimen was regarded as negative.

On the basis of an "expanded" gold standard, defined as a positive wet mount and PCR test with primers TVK3 and TVK7 and/or a positive PCR test confirmed by a second PCR assay with primers TVA5-1 and TVA6, the overall prevalence of trichomoniasis in the population studied was 12.6% (54 of 428). The rate of detection of *T. vaginalis* was 13.3% (57 of 428) by PCR, 6.1% (26 of 428) by wet mount, and 8.4% (36 of 428) by the Xenostrip-Tv test. Fifty-four of the 57 specimens that were positive by PCR were confirmed by a second PCR assay with primers TVA5-1 and TVA6 (data not shown). DNA was re-extracted from the three specimens that showed discordant results and retested with a PCR assay with primers TVK3 and TVK7, and these specimens remained positive. Overall, 18 specimens were positive by PCR alone. The sensitivities and specificities of wet mount, PCR, and the Xenostrip-Tv test compared to those of the expanded gold standard are shown in Table 1. The Xenostrip-Tv sensitivity and specificity were 100 and 97.5% compared to those of wet mount because the Xenostrip-Tv assay detected 10 additional specimens ($P < 0.0001$, Fisher's exact test). The Xenostrip-Tv sensitivity and specificity were 66.6 and 100% compared to those of the expanded gold standard. The sensitivity and specificity of wet mount compared to those of the gold standard were 48.1 and 100%.

T. vaginalis infection is very common in the United States and has been shown to be associated with adverse pregnancy outcomes. Despite this, very little or no emphasis has been placed on improving the diagnosis of *T. vaginalis* infection, which largely depends on microscopy of wet preparations or, less frequently, on culture of trichomonads.

With an expanded gold standard, we have shown the prevalence of *T. vaginalis* infection to be 12.6% (54 of 428) in our study population, compared to 6 and 8.4% by wet mount and Xenostrip tests, respectively. Microscopic examination of a wet mount is widely used for the rapid diagnosis of *T. vaginalis* infections. The specificity of this test in women is usually high; however, as has been shown in this study, compared to PCR, the sensitivity is very poor, with reported rates varying from 34.2 to 58.5% (8, 9, 10, 13, 18). Culture remains the gold

standard for the diagnosis of trichomoniasis, but this method requires a laboratory and specialized equipment for growing and identifying *T. vaginalis*. It is not a practical diagnostic method in sexually transmitted disease clinics, adult health centers, and physicians' offices, where there is a rapid turnaround of patients. A rapid diagnostic test that does not require special equipment or a formal laboratory is needed in these settings. The InPouch TV test (1), which is a disposable culture system for the maintenance, transport, and detection of *T. vaginalis*, is an improvement on routine culture in Diamond's or Trichosel medium; however, an incubator and a microscope are still required. In addition, varying transport times from primary health care settings may cause evaporation of the fluid in the InPouch medium, resulting in loss of organisms and thus loss of sensitivity. The Xenostrip-Tv test, on the other hand, does not require sophisticated equipment and can be performed on site by laboratory personnel, nurses, and medical practitioners with minimal training.

The sensitivity of the Xenostrip-Tv test compared to that of PCR was low; however, it was significantly more sensitive than wet mount, which is routinely used in clinics throughout the United States and elsewhere. In addition, the test did not detect any false positives compared to the expanded gold standard defined in this study, it was found to be reliable and easy to perform, and results were available within 10 min. Recently, the Xenostrip-Tv test was compared to InPouch TV with 20 InPouch TV culture-positive and 40 randomly selected culture-negative vaginal swab specimens. In this small evaluation, a sensitivity and specificity of 90 and 92.5% were demonstrated (11). These authors stated that the sensitivity and specificity of the Xenostrip test compared to InPouch TV culture are lower than the 100% sensitivity and 98.1% specificity stated by the manufacturer in the package insert. However, the sensitivity and specificity reported by the manufacturer were based on a comparison between Xenostrip and wet mount and only discordant results were resolved by culture. In our study, the sensitivity and specificity of the Xenostrip test compared to wet mount were 100 and 97.5%, which are similar to those reported by the manufacturer. Although the authors have shown that the Xenostrip test is less sensitive and specific than culture with InPouch TV, it is being marketed as a rapid point-of-care test and therefore should be compared to wet mount unless it is intended to replace InPouch TV or culture in Diamond's medium in a particular setting.

In this study, three specimens that were positive by PCR with primers TVK3 and TVK7 tested negative by a confirmatory PCR. These specimens tested positive when the PCR with primers TVK3 and TVK7 was repeated on a separate occasion. This assay also incorporates carryover prevention, which rules out the possibility of amplicon contamination. Furthermore, it has recently been shown (5) that the sensitivity of different PCR assays for the diagnosis of *T. vaginalis* infections varies, with primers TVK3 and TVK7 and primers TVA5 and TVA6 displaying sensitivities of 87.3 and 74.7%. In this study, primer TVA5-1 was used instead of TVA5 to prevent primer-dimer formation during PCR amplification (9). The sensitivity of PCR assays with these primers in combination with TVA6 should be about the same. Taking all of these factors into consideration, it is unlikely that the test results for these three specimens were false positive.

Our results confirm reports by other workers (14, 17) that PCR with vaginal swab specimens is more sensitive than wet mount for the detection of *T. vaginalis*. However, PCR is not a viable alternative in settings where rapid tests such as Xenostrip-TV and wet mount are used. In this study, we have shown that the Xenostrip-Tv test for *T. vaginalis* is more sensitive than the routinely used wet mount test when using vaginal swab specimens and it is rapid and easy to perform. This assay may be useful in settings where a rapid point-of-care test is needed or where microscopy and culture are impractical since the test result is easily read and does not require any expensive instrumentation. Studies to evaluate the use of the Xenostrip-TV test with noninvasive specimens such as first-catch urine should be considered. However, it is anticipated that there would be a loss of sensitivity since the vaginal wall is the prime source of infecting trichomonads and detection in urine would depend largely on the degree of urinary contamination with vaginal secretions.

We sincerely thank Theresia Snelling, Hanan Debabneh, and Jalaja Siddappa from the Dekalb Central, Kirkwood, and Richardson Adult Health Centers, respectively, in Atlanta, Ga., for performing wet mount testing and providing clinical specimens for this study. We are grateful to Evan Secor, CDC, for generously providing laboratory isolates of *T. vaginalis*.

Use of trade names is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention. We do not have any financial or other interest in either Xenotope Diagnostics or Genzyme Diagnostics.

REFERENCES

1. **Borchardt, K. A., and R. F. Smith.** 1991. An evaluation of an InPouch™ TV culture method for diagnosing *Trichomonas vaginalis* infection. Genitourin. Med. **67:**149–152.
2. **Cates, W., Jr.** 1999. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. Sex. Transm. Dis. **26:**S2–S7.
3. **Cohen, M. S.** 1998. Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis. Lancet **351**(Suppl. III)**:**5–7.
4. **Cotch, M. F., J. G. Pastorek II, R. P. Nugent, S. L. Hillier, R. S. Gibbs, D. H. Martin, et al.** 1997. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. Sex. Transm. Dis. **24:**353–360.
5. **Crucitti T., E. Van Dyck, S. Abdellati, and M. Laga.** 2001. Comparison of polymerase chain reaction techniques for the diagnosis of *Trichomonas vaginalis*. Int. J. STD AIDS **12**(Suppl. 2)**:**37.
6. **Kengne, P., F. Veas, N. Vidal, J.-L. Rey, and G. Cuny.** 1994. *Trichomonas vaginalis*: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. Cell. Mol. Biol. **40:**819–831.
7. **Laga, M., A. Manoka, M. Kivuvu, B. Malele, M. Tuliza, N. Nzila, et al.** 1993. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS **7:**95–102.
8. **Lawing, L. F., S. R. Hedges, and J. R. Schwebke.** 2000. Detection of trichomoniasis in vaginal and urine specimens from women by culture and PCR. J. Clin. Microbiol. **38:**3585–3588.
9. **Madico, G., T. C. Quinn, K. Rompalo, T. McKee, Jr., and C. A. Gaydos.** 1998. Diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swab samples. J. Clin. Microbiol. **36:**3205–3210.
10. **Mahmoud, M. S., S. S. Abdel-Aziz, E. A. El-Sherif, and K. H. Swidan.** 1999. Diagnosis of symptomatic and asymptomatic *Trichomonas vaginalis* infection by applying one tube nested PCR to vaginal discharge. J. Egypt. Soc. Parasitol. **29:**1031–1046.
11. **Miller, G. A., J. D. Klausner, T. J. Coates, R. Meza, C. A. Gaydos, J. Hardick, S. Leon, and C. F. Caceres.** 2003. Assessment of a rapid antigen detection system for *Trichomonas vaginalis* infection. Clin. Diagn. Lab. Immunol. **10:**1157–1158.
12. **Pillay, A., H. Liu, C. Y. Chen, B. Holloway, A. W. Sturm, B. Steiner, and S. A. Morse.** 1998. Molecular subtyping of *Treponema pallidum* subspecies *pallidum*. Sex. Transm. Dis. **25:**408–414.
13. **Rampalo, A. M., C. A. Gaydos, N. Shah, M. Tennant, K. A. Crotchfelt, G. Madico, T. C. Quinn, R. Daniel, K. V. Shah, J. C. Gaydos, and K. T. McKee, Jr.** 2001. Evaluation of use of a single intravaginal swab to detect multiple sexually transmitted infections in active-duty military women. Clin. Infect. Dis. **33:**1455–1461.

14. **Ryu, J.-S., H.-L. Chung, D.-Y. Min, Y.-H. Cho, Y.-S. Ro, and S.-R. Kim.** 1999. Diagnosis of trichomoniasis by polymerase chain reaction. Yonsei Med. J. **40:**56–60.
15. **Schwebke, J. R., and L. F. Lawing.** 2002. Improved detection by DNA amplification of *Trichomonas vaginalis* in males. J. Clin. Microbiol. **40:**3681–3683.
16. **Schwebke, J. R., and E. W. Hook III.** 2003. High rates of *Trichomonas vaginalis* among men attending a sexually transmitted diseases clinic: implications for screening and urethritis management. J. Infect. Dis. **188:**465–468.
17. **van der Schee, C., A. van Belkum, L. Zwijgers, E. van der Brugge, E. L. O'Neill, A. Luijendijk, T. van Rijsoort-Vos, W. I. Van der Meijden, H. Verbrugh, and H. J. F. Sluiters.** 1999. 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. **37:**4127–4130.
18. **Wendel, K. A., E. J. Erbelding, C. A. Gaydos, and A. M. Rompalo.** 2002. *Trichomonas vaginalis* polymerase chain reaction compared with standard and therapeutic protocols for detection and treatment of vaginal trichomoniasis. Clin. Infect. Dis. **35:**576–580.
19. **World Health Organization.** 1995. An overview of selected curable sexually transmitted diseases, p. 2–27. *In* Global program on AIDS. World Health Organization, Geneva, Switzerland.