JOURNAL OF CLINICAL MICROBIOLOGY, July 2004, p. 2940–2943 Vol. 42, No. 7
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.7.2940–2943.2004

Performance of a New, Rapid Assay for Detection of *Trichomonas vaginalis*

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Received 16 November 2003/Returned for modification 12 March 2004/Accepted 5 April 2004

***Trichomonas vaginalis* infection is highly prevalent, may have serious health consequence, and is readily treatable. However, screening has been limited by currently available tests, which tend to be insensitive, expensive, or require a delay before results are reported. The XenoStrip-Tv (Xenotope Diagnostics, Inc., San Antonio, Tex.) was evaluated on vaginal swab specimens from 936 women attending sexually transmitted disease clinics in Seattle, Wash. ($n = 497$), and Birmingham, Ala. ($n = 439$). *T. vaginalis* prevalence by culture (InPouch; Biomed) was 8.7% in Seattle and 21.0% in Birmingham. Compared to culture, the XenoStrip assay in Seattle was 76.7% (95% confidence interval [95% CI] = 61.4 to 88.2) sensitive and 99.8% (95% CI = 98.8 to 99.9) specific, and in Birmingham it was 79.4% (95% CI = 69.6 to 87.1) sensitive and 97.1% (95% CI = 94.8 to 98.6) specific. The positive predictive values were 97.1% in Seattle and 87.9% in Birmingham; the negative predictive values were 97.8 and 94.7%, respectively. Rapid test performance did not vary by vaginal symptoms or by the presence of other vaginal or cervical syndromes or infections. The sensitivity did vary by day of culture-positive result, with a 71% decline in XenoStrip sensitivity for every additional day delay until *T. vaginalis* was first detected in cultures (odds ratio = 0.29, 95% CI = 0.18 to 0.49). The rapid assay was more sensitive than wet preparation microscopy (78.5% versus 72.4% [$P = 0.04$]) but was less specific (98.6% versus 100% [$P = 0.001$]). The XenoStrip rapid assay is well suited for use in settings with a moderately high prevalence of *T. vaginalis* infection, particularly when microscopy is not practical.**

Trichomoniasis is the most common nonviral sexually transmitted infection (STI) globally and in the United States (10). Although not a reportable STI in the United States, an estimated five million women and one million men are infected annually with *Trichomonas vaginalis*, a common cause of vaginitis and urethritis (3, 6). The prevalence in women has ranged from 2% in low-risk populations to 60% in the highest-risk groups (10). As many as 80% of women with trichomoniasis have been asymptomatic in some settings (1), and, in the absence of treatment, infection is thought to persist for years (2). *T. vaginalis* infection has been associated with adverse pregnancy outcomes (4, 9, 16) and with increased human immunodeficiency virus (HIV) shedding in women infected with both *T. vaginalis* and HIV (13).

Although culture is the "gold standard" for the diagnosis of trichomoniasis, it is not widely available (8). Nucleic acid amplification has been shown to have high sensitivity but also is not widely available (5). Microscopic examination of vaginal secretions (wet preparation) is the most frequently used diagnostic test for trichomoniasis; however, test sensitivity depends greatly on examiner expertise, and access to a microscope is required.

We evaluated here the performance of a new rapid point-of-care test for *T. vaginalis*, XenoStrip-Tv (Xenotope Diagnostics, Inc., San Antonio, Tex.) in comparison with culture and wet preparation for the diagnosis of trichomoniasis in women.

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MATERIALS AND METHODS

Design and specimen collection. Women were recruited at public sexually transmitted disease clinics in Seattle, Wash., and Birmingham, Ala. Women were eligible if they were ≥14 years of age, were to receive a pelvic examination, and were able to give verbal consent in English. The Human Subjects Review Committees at the University of Washington and the University of Alabama at Birmingham approved the study protocol.

Women were enrolled from November 2001 through February 2002 in Birmingham and from January 2002 through June 2002 in Seattle. After collection of a vaginal swab for wet preparation microscopy and before collection of endocervical specimens for gonorrhea and chlamydia tests, two additional swabs—one for the XenoStrip assay and one for culture—were collected by using sterile Dacron swabs from vaginal fluid pooling in the lateral fornices. The order of study swab collection was randomly assigned either by study identification number (Seattle) or to each collecting clinician (Birmingham).

Laboratory. For the wet preparation microscopy, one vaginal swab was mixed with 0.5 ml of normal saline immediately, and a drop of the mixture was placed on a slide and examined under a microscope at ×200 magnification. In Seattle, wet preparations were read by each of the clinicians as part of routine participant evaluation. In Birmingham, the wet preparations were read by one of three research nurses.

The XenoStrip test uses color immunochromatographic, capillary flow dipstick technology to detect the presence of stable *T. vaginalis* antigens from vaginal samples, utilizing immunoglobulin G1 monoclonal antibodies XDTv1 and XDTv2 to target intracellular and surface secretory proteins. *T. vaginalis* proteins are extracted from swab specimens, and antigen-antibody binding results in a red line on the test strip. A second red line serves as an internal positive control for test performance. Sample swabs were placed into plastic tubes and stored at 4°C until processed within 24 h of collection. Although the XenoStrip is a rapid point-of-care test, the testing for the present study was performed in the laboratory to standardize test comparison. The test was performed according to the manufacturer's instructions. The vaginal swab was placed in a microfuge tube

TABLE 1. Comparison of XenoStrip rapid test performance to *T. vaginalis* culture in Seattle and Birmingham in 2002[a]

Site	Prevalence (%)[b]	No. true positive	No. false positive	No. false negative	No. true negative	Sensitivity		Specificity		Predictive value (%)			
										Positive		Negative	
						%	95% CI	%	95% CI	%	95% CI	%	95% CI
Seattle	43/497 (8.65)	33	1	10	453	76.7	61.4–88.2	99.8	98.8–99.9	97.1	84.7–99.9	97.8	96.1–98.9
Birmingham	92/439 (20.96)	73	10	19	337	79.4	69.6–87.1	97.1	94.8–98.6	87.9	78.9–94.1	94.7	91.8–96.8
Total	135/936 (14.42)	106	11	29	790	78.5	70.6–85.1	98.6	97.6–99.3	90.6	83.8–95.2	96.5	94.9–97.6

[a] The gold standard is trichomonads visualized in culture.
[b] That is, the number of positive samples/the total number of samples tested.

containing 0.5 ml of sample buffer (phosphate-buffered saline [pH 7.4] containing 0.5% Triton X-100 and 0.01% NaN_3) and mixed for 1 min, and the solution was expressed from the swab. The XenoStrip test strip was placed into expressed specimen and read at 10 min, and initially negative specimens were read again at 20 min. External positive control samples were used to validate the XenoStrip weekly.

The InPouch (Biomed, San Jose, Calif.) system was used for *T. vaginalis* culture. Specimens were processed according to the manufacturer's instructions and were examined for characteristic trichomonad morphology and motility at ca. 24 h and then every other day for a maximum of 7 days after inoculation.

In Seattle, two laboratory technicians read all XenoStrip assays and cultures. One reader assessed approximately two-thirds of all of the rapid assays, and the other read two-thirds of the cultures; both readers were blinded to the results of the other test for each study subject and to the wet preparation results. These readers then switched which assay they read. The results were recorded in separate lab notebooks and compiled and analyzed by a third staff person. In Birmingham, three research nurses read the rapid assays and the wet preparations and were not blinded to the results of each test. Cultures were read by laboratory staff, who were blinded to the rapid antigen test results.

Analysis. Study results were merged with relevant demographic and clinical data from the participant's medical record and analyzed by using STATA 7.0 (STATA Corp., College Station, Texas). The study population within and between sites and test performance differences by symptoms, coinfections, or swab order were compared by using chi-square or Fisher exact tests for dichotomous variables and parametric or nonparametric tests as appropriate for continuous variables. Assessment of XenoStrip sensitivity by day of culture positivity was conducted by using logistic regression on the subset of all culture-positive cases. Comparison of rapid assay and microscopy performance utilized McNemar's chi-square test.

RESULTS

In Seattle 509 women were enrolled, with 12 exclusions for a total of 497 available for analysis. Exclusions occurred because of culture refrigeration (one), a missing XenoStrip swab (one) or testing more than 24 h after collection (four), and the use of a different reagent lot (six). By culture, 43 (8.7%) of 497 women were positive for *T. vaginalis*. In Birmingham 439 women were enrolled and 92 (21.0%) were found to be positive by culture.

Compared to women in Birmingham, enrolled Seattle women were somewhat older (mean age of 29 years versus 28 years [$P = 0.02$]), were more likely to identify themselves as white (51.3% versus 11.4% [$P < 0.001$]), and were less likely to report vaginal discharge, itching, or dysuria (47.3% versus 72.5% [$P < 0.001$]). Combining both sites, 59% of women had vulvovaginal symptoms and 53% of women identified themselves as African-American.

Compared to culture, the sensitivity of the XenoStrip assay was similar in Seattle (76.7%) and Birmingham (79.4%); the specificity was somewhat lower in Birmingham (97.1%) than in Seattle (99.8% [$P < 0.01$]) (Table 1). Overall, the prevalence of *T. vaginalis* was 14.4%, the sensitivity of the rapid test was 78.5% (95% confidence interval [CI] = 70.6 to 85.1), and the specificity was 98.6% (95% CI = 97.6 to 99.3).

XenoStrip assay sensitivity varied by the number of days until *T. vaginalis* was first detected in cultures. Excluding cultures for which 24-h results could not be determined over the weekend, the time to first positive culture did not differ between the two sites ($P = 0.57$). Overall, the XenoStrip assay correctly identified as positive 88 (93.7%) of 94 specimens found to be positive by culture at day 1, 9 (45.0%) of 20 specimens positive by culture at day 2, 7 (58.3%) of 12 specimens positive by culture at day 3, and 2 (22.2%) of 9 specimens positive by culture at ≥4 days after inoculation (test for linear trend, $P < 0.001$). In a logistic regression excluding cultures collected on Fridays, the association between day of first culture positive result and XenoStrip positivity was an odds ratio of 0.29 (95% CI = 0.18).

The sensitivity and specificity of the XenoStrip assay did not vary between women who reported vaginal discharge, itching, or dysuria (e.g., a sensitivity of 77.8% with reported vaginal discharge versus a sensitivity of 79.1% with no reported discharge [$P = 0.53$]). XenoStrip assay performance did not vary by presence of other syndromes or pathogens, including diagnosis of bacterial vaginosis or infection with *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. Test performance also did not vary by swab order.

Compared to culture results, the wet preparation sensitivity in Seattle was 66.7% (95% CI = 50.5 to 80.4%) and the specificity was 100% (95% CI = 99.2 to 100.0%). In Birmingham, the wet preparation sensitivity was 75% (95% CI = 64.9 to 83.5%) and the specificity was 100% (95% CI = 98.9 to 100.0%). Of 135 women with positive cultures in either study site, only 97 (71.8%) were diagnosed with trichomoniasis by microscopy, permitting treatment on the day of visit. Of the remaining 38 culture-positive women who had a negative or missing wet preparation result, 23 were treated with metronidazole regimens, usually for other clinical syndromes such as bacterial vaginosis. This left 15 women who were neither diagnosed nor appropriately treated on the day of study visit. Five of these fifteen women (33.3%) were XenoStrip positive. The XenoStrip correctly identified 11 (28.9%) of the 38 women with positive cultures but negative or missing wet preparations while incorrectly identifying as positive 11 culture-negative women (1 in Seattle and 10 in Birmingham). Overall, the XenoStrip rapid assay was statistically significantly more sensitive than wet preparation microscopy (78.5% versus 72.4% [$P = 0.04$]) but was less specific than wet preparation (98.6% versus 100.00% [$P = 0.001$]).

DISCUSSION

Trichomoniasis is one of the most common STIs and has been associated with important sequelae (4, 9, 11, 15–17; P. Kissinger, A. Amedee, R. A. Clark, J. Dumestre, M. Magnus, and T. A. Farley, unpublished data [presented at the 15th ISSTDR Conference, Ottawa, Canada, August 2003]). Although many clinics that perform tests for *T. vaginalis* restrict testing to symptomatic women, studies associating *T. vaginalis* with HIV shedding have prompted reconsideration of the pathogen's importance, and some authorities have called for more widespread screening and treatment (2, 7, 14). Screening is enhanced when an accurate test is available for a treatable disease.

Currently available tests have limitations that inhibit widespread testing and may contribute to the underdiagnosis of trichomoniasis. Culture is considered to be the reference test (12) for *T. vaginalis* but is not widely available, and specimens must be examined for up to 7 days until being considered negative. Many clinical facilities continue to utilize wet preparation microscopy, which even among skilled diagnosticians has been shown to have a sensitivity of only 50 to 80% compared to culture (18). Moreover, beyond its evident diagnostic limitations for many settings, wet preparation assessment is not an option, as it requires a microscope which today is not frequently available in clinics. This is particularly true in resource-poor settings. PCR assays have been shown to be sensitive but, like culture, are not widely available and do not provide results at the time of clinical assessment.

Women who are identified as infected on the day of their clinic visit are more likely to receive and to comply with treatment. A point-of-service assay conveys screening and management advantages, particularly if it performs well among asymptomatic women. For possible use as a screening test, it is encouraging that the sensitivity of the XenoStrip assay did not differ between symptomatic and asymptomatic women.

Our study found that the XenoStrip assay performs as well or better than wet preparation microscopy interpreted by experienced clinicians, and makes rapid *T. vaginalis* identification available in settings without microscopy. As such it enhances the diagnostic options currently available to clinicians and researchers beyond the mainstays of microscopy, culture, or PCR. The XenoStrip assay and wet mount microscopy require similar personnel time to interpret; however, the results of wet mount microscopy often are available more quickly. Unlike culture, this 10 to 20 min assay does allow same-day results to be given.

The difference in the specificity of the XenoStrip assay between the Seattle and Birmingham sites remains unexplained. Test protocols were identical at the two clinical sites and, interestingly, *T. vaginalis* prevalence was 2.5-fold greater at the site with lower XenoStrip assay specificity. It is possible that the persons at the two clinical sites adopted subtle differences in the colorimetric thresholds used to define a positive test or that there were undetected differences in the sensitivity of culture at the two sites. The observed specificity of 97.1% at the Birmingham site may be adequate for use of the test in populations of moderate or high prevalence. In low-prevalence populations, further evaluation of the specificity of the XenoStrip assay should be considered before widespread use can be recommended.

In summary, the XenoStrip is a simple-to-use, rapid diagnostic test that does not require either specialized training or equipment. Expert microscopy requires equipment and upkeep costs, as well as technician training for accurate diagnostic skills. The XenoStrip has a sensitivity comparable to or better than expertly performed wet preparation and a specificity adequate for use in moderate- to high-prevalence settings. More widespread use could facilitate *T. vaginalis* screening and more-accurate evaluation of women with vaginal discharge.

ACKNOWLEDGMENTS

This study was funded by Xenotope Diagnostics, Inc. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. Clinical study agreements at the University of Washington and the University of Alabama adhered to guidelines for company-sponsored clinical research.

We thank John Alderete, Jr., for support. We are grateful for the participation of University of Washington and University of Alabama patients, clinicians, and laboratory staff, particularly Karen Winterscheid.

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