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Use of an Immunochromatographic Assay for Rapid Detection of *Trichomonas vaginalis* in Vaginal Specimens

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***Trichomonas vaginalis* infection is estimated to be the most widely prevalent nonviral sexually transmitted infection in the world. Wet-mount microscopy is the most common diagnostic method, although it is less sensitive than culture. The OSOM Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, Mass.) (referred to here as OSOM) is a new point-of-care diagnostic assay for *T. vaginalis* that uses an immunochromatographic capillary flow (dipstick) assay and provides results in 10 min. The purpose of this study was to determine the test characteristics of OSOM compared to those of a composite reference standard (CRS) comprised of wet-mount microscopy and *T. vaginalis* culture. This multicenter cross-sectional study enrolled sexually active women ≥18 years of age who presented with symptoms of vaginitis, exposure to *T. vaginalis*, or multiple sexual partners. Vaginal-swab specimens were obtained for *T. vaginalis* culture, wet mount, and rapid testing. The prevalence of *T. vaginalis* in this sample was 23.4% (105 of 449) by the CRS. The sensitivity and specificity of OSOM vaginal-swab specimens were 83.3 and 98.8%, respectively, while wet mount had a sensitivity and specificity of 71.4 and 100%, respectively, compared to the CRS. OSOM performed significantly better than wet mount ($P = 0.004$) and detected *T. vaginalis* in samples that required 48 to 72 h of incubation prior to becoming culture positive. The performance of the rapid test was not affected by the presence of coinfections with chlamydia and gonorrhea. The OSOM Trichomonas Rapid Test is a simple, objective test that can be expected to improve the diagnosis of *T. vaginalis*, especially where microscopy and culture are unavailable.**

Trichomonas vaginalis infection is estimated to be the most widely prevalent nonviral sexually transmitted infection (STI) in the world. Weinstock et al. estimated that ~7.4 million new cases of *T. vaginalis* infection occurred in 2000 in the United States, compared to 2.8 million cases of *Chlamydia trachomatis* infection and 718,000 cases of *Neisseria gonorrhoeae* infection (16). The prevalence of *T. vaginalis* is likely to be underestimated because there are no guidelines for *T. vaginalis* screening of women, and clinicians often rely upon insensitive diagnostic methods.

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 (17).

The present "gold standard" for the diagnosis of *T. vaginalis* is culture. Selective media for culture include Diamond's, Trichosel, and InPouch TV (BioMed Diagnostics, San Jose, Calif.). No single culture appears to have 100% sensitivity for *T. vaginalis* infection. When all three media are inoculated with a known-positive sample, all of the culture media are comparable (85 to 87% sensitive and 100% specific), when the gold standard is defined as growth of *T. vaginalis* in at least one of the three media (1, 11). The advantages of the InPouch culture include its ease of use; the combination of specimen transport, growth, and evaluation; and its long shelf life. However, it requires on-site incubators, microscopes, and 24 to 120 h for final results. The only commercially available product is a nucleic acid probe test (AffirmVPIII; Becton Dickinson, Sparks, Md.). It has excellent sensitivity (90%) but requires 45 min for results, is more expensive than culture, and is classified as a moderately complex test (4, 6). Other nucleic acid amplification techniques for *T. vaginalis* available for research studies appear to have excellent sensitivity and specificity but are not applicable for point-of-care use (7). One immunochromatographic monoclonal-antibody-based detection system (Xeno-Strip-Tv; Xenotope Diagnostics, Inc., San Antonio, Tex.) has demonstrated a sensitivity of 78.5 to 90% when performed on vaginal-swab samples, but it requires up to 20 min to read (9, 12).

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The OSOM Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, Mass.) is a new point-of-care diagnostic assay for *T. vaginalis*. Like the XenoStrip-Tv, it is an immunochromatographic capillary flow (dipstick) assay. The test employs a pair of murine monoclonal antibodies, one immobilized on the surface of the dipstick and the other conjugated to particles and dried onto the dipstick. In the present study, we determined the sensitivity and specificity of the OSOM Trichomonas Rapid Test compared to those of a composite reference standard (CRS) of wet-mount microscopy and culture performed on two types of samples: vaginal swabs and saline solution remaining after wet-mount microscopy.

MATERIALS AND METHODS

Study population. Sexually active women 18 years of age or older presenting with signs and symptoms of vaginosis or vaginitis or due to exposure to *T. vaginalis* through an infected partner or high-risk behavior were enrolled in a prospective, cross-sectional, multicenter study. High-risk behavior was defined as two or more sexual partners reported within the last 30 days. Patients were also admitted into the study if they presented for a follow-up visit because of a previously diagnosed *T. vaginalis* infection and were within 30 days posttreatment. Study clinicians at eight participating sites informed eligible patients about the study and enrolled those who consented to participate. The study was approved by the institutional review board at each research center. Enrollment occurred between June and October 2003, ending when the target sample size of 100 *T. vaginalis*-positive patients had been reached.

Collection of specimens. Following a history and physical examination, the clinicians obtained three vaginal samples from each patient by swabbing the vaginal vault. The clinicians were asked to vary the order in which they obtained the vaginal swabs to prevent sampling bias. One swab was placed in a plastic tube containing 0.5 ml of saline and used immediately for wet-mount microscopy. Another swab was used to immediately inoculate an InPouch TV culture device. A third swab was placed in a 12- by 75-mm dry plastic tube and stored frozen at or below −20°C. In addition, the remainder of the saline solution used to prepare the wet mount (wet-mount saline) was also stored frozen. Dry swabs and wet-mount saline samples without identification were shipped to the Genzyme Corporation on dry ice by overnight courier at scheduled intervals. The specimens were stored frozen for up to 17 weeks prior to being tested. Additional cervical samples were obtained for detection of other sexually transmitted diseases (STDs), such as *C. trachomatis* and *N. gonorrhoeae* infections, according to the local standard of care. At six sites, every enrollee was tested for *N. gonorrhoeae* infection by culture or Pace2 (GenProbe, San Diego, Calif.). At five sites, every enrollee was tested for *C. trachomatis* infection by Pace2 or ProbetecET (Becton Dickinson, Sparks, Md.).

Laboratory methods. Wet-mount microscopy was performed according to the standard of care at each site. In general, a swab containing the vaginal sample was placed in a 12- by 75-mm plastic tube containing 0.5 ml of saline. Clinicians or laboratory technicians transported the swab to an in-office laboratory. After vigorous mixing of the swab in the saline, the swab was removed and depressed onto a clean, dry microscope slide to express a small amount of liquid. A coverslip was placed over the sample, and the slide was examined with a bright-field microscope by the clinician or laboratory technician within 10 min of collection. For culture, the InPouch TV system was performed according to the manufacturer's instructions. Briefly, the InPouch TV system was inoculated by placing a vaginal-swab sample into the upper chamber, mixing the swab in the medium, and then discarding the swab and sealing the pouch. The contents of the upper chamber were immediately expressed into the lower chamber, and the pouch was sealed and placed into a 37°C incubator. The culture was then read in the same manner as a wet mount for up to 5 days or until it was positive.

In order to provide standardization for this research study, the OSOM Trichomonas Rapid Test was performed on batched samples in the Genzyme research laboratory over a 12-day testing period. Operators were blinded to wet-mount and *T. vaginalis* culture results. Swab specimens were allowed to warm to room temperature before testing began. Using the supplied dropper top, 0.5 ml of sample buffer was added to flexible plastic test tubes supplied with the kit. Swabs containing vaginal specimens were placed in the tubes containing sample buffer, mixed vigorously, and allowed to sit for ~1 min. Excess liquid was removed from the swabs by squeezing the sides of the test tubes, and the swabs were removed and discarded. An OSOM Trichomonas Rapid Test stick was placed in each tube containing the buffer-sample mixture, and results were read at 10 min. A positive result was indicated by the presence of a blue test line along with a red control line, whereas in a negative result, only a red control line was visible. If no red control line was visible, the test result was considered invalid. To test the wet-mount saline solution, a clean swab was inserted into the thawed wet-mount saline and placed in a flexible plastic test tube containing sample buffer, and the test was performed as previously described.

Analysis. The results of OSOM tests of the vaginal swab and the wet-mount saline were compared individually to those of *T. vaginalis* culture, wet mount, and the CRS. The CRS was defined as positive if there was a positive result by either wet mount or culture. Samples were classified as negative if both wet mount and culture were negative. Comparisons of the difference in sensitivities between wet mount and OSOM were done using McNemar's test for paired samples. All analyses were performed using Excel (Microsoft Corp., Redmond, Wash.), JMP (SAS Institute, Cary, N.C.), and Stata version 7 (StataCorp, College Station, Tex.).

RESULTS

A total of 511 patients were enrolled in the study at seven sites throughout the United States. One site was excluded from the study because all 62 swabs collected for the rapid test were inadvertently discarded by the site. For the remaining 449 patients enrolled, 439 vaginal swabs were received for OSOM testing; 2 gave invalid test results, leaving 437 for analysis. Four hundred forty-eight samples of wet-mount saline were received for OSOM testing (1 gave invalid test results, leaving 447 for analysis). Wet mounts were read for 449 patients, and 405 vaginal-swab cultures were read for 5 days (or until they were positive). Additional testing was performed for *N. gonorrhoeae* in 415 patients and *C. trachomatis* in 315 patients.

Nearly all subjects (439 of 449; 98%) who enrolled presented to a study site with signs and symptoms of vaginitis or vaginosis. Among these 439 symptomatic subjects, 95 (22%) also reported high-risk behavior (i.e., >2 sex partners in the last 30 days), and 23 (5%) also reported contact with an infected partner. Four subjects met all three enrollment criteria. Four subjects presented for a follow-up visit with signs and symptoms. Ten subjects did not exhibit signs or symptoms; six were eligible due to high-risk behavior alone, and four had an infected partner.

Overall, the prevalence of *T. vaginalis* in this population was 23.4% (105 of 449) by the CRS and was similar across sites (range, 17.2 to 28.7%). African-Americans were the predominant racial group represented (76%), followed by Caucasian (21%), Native American (2.7%), other (2.2%), Hispanic (4%), and Asian (0.4%). Some patients listed more than one racial background, resulting in a total greater than 100%. The mean age for all patients was 28 years old (range, 18 to 61 years old). The prevalence of *T. vaginalis* was higher in women over age 30 than in those aged 18 to 29 (31.3 versus 19.2%; $\chi^2 = 8.2$; $P = 0.004$).

The study results are displayed in Fig. 1 and Table 1. In comparison to the CRS, the sensitivity and specificity of OSOM were 83.3 and 98.8%, respectively, while wet mount was 71.4% sensitive. The sensitivity of the rapid test was statistically superior to that of wet mount ($P = 0.004$). By definition, the specificity of wet mount relative to the CRS is 100% (Table 1). Compared to culture alone, OSOM was 83.5% sensitive and 99.0% specific, whereas wet mount was 70.0% sensitive and 99.7% specific. The positive and negative predictive values of the rapid test were 95.5 and 95.1%, respectively. Among the specimens tested by rapid test, culture, and wet

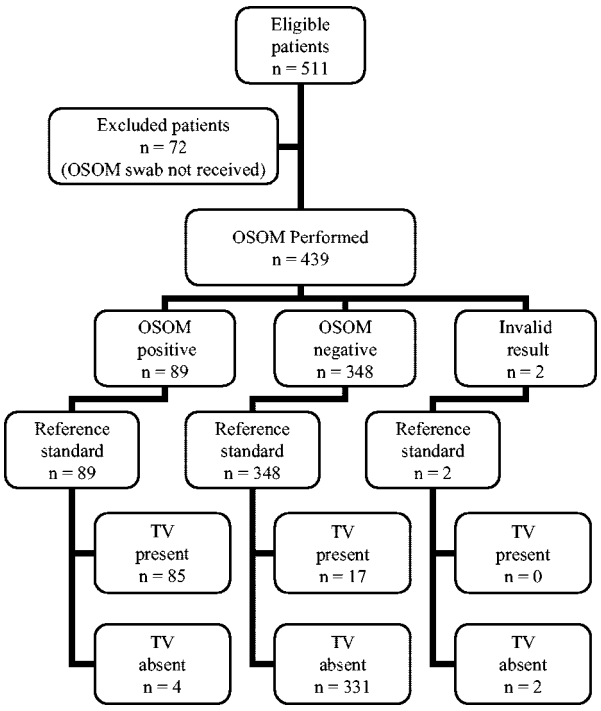

FIG. 1. Study flow diagram. TV, *T. vaginalis.*

mount, OSOM detected 11 positive samples missed by wet mount (Table 2). The more favorable sensitivity of the rapid test compared to wet mount is due in part to the ability of OSOM to detect *T. vaginalis* in samples that did not yield a positive culture for 48 to 72 h. Both the rapid test and wet mount missed one specimen that became positive after 4 days of culture and one specimen that became positive after 5 days of culture.

Because wet-mount microscopy is inexpensive and can detect other infections, such as bacterial vaginosis and yeast, we examined the performance of wet mount and the rapid test for a single vaginal sample. Wet-mount saline tested with OSOM demonstrated a sensitivity of 75.2% and a specificity of 98.5% compared to the CRS, similar to the test characteristics of wet mount compared to those of the CRS. However, the rapid test of wet-mount saline detected an additional 27% (8 of 30) of *T. vaginalis* infections that were wet mount negative but culture positive.

To determine if coinfections had an effect on the rapid test, we evaluated the sensitivity and specificity of OSOM compared to those of the CRS stratified by coinfection status. To do this, we looked at the subset of subjects that were tested for both *N. gonorrhoeae* and *T. vaginalis* ($n = 415$) and at the subset tested for both *C. trachomatis* and *T. vaginalis* ($n = 315$). In patients with a concurrent *C. trachomatis* infection, the sensitivity of the rapid test was 85.7% compared to 80.9% in those with a negative *C. trachomatis* test. Similarly, in patients coinfected with *N. gonorrhoeae*, the OSOM sensitivity was 76.9% compared to 83.7% in those with a negative *N. gonorrhoeae* test. The specificity of the rapid test did not differ significantly by *C. trachomatis* infection status (100 versus 98%) or by *N. gonorrhoeae* infection status (94.7 versus 99%). Due to the small numbers, Bayesian analysis was performed to test the hypothesis that the sensitivity and specificity did not differ by >10%. These differences were not statistically significant.

TABLE 2. OSOM- and wet-mount-positive samples stratified by day culture positive

Day culture positive	No. of specimens positive by:		
	Culture	OSOM	Wet mount
1	76	73	69
2	13	6	1
3	6	2	0
4	1	0	0
5	1	0	0

DISCUSSION

Historically, *T. vaginalis* has been viewed as a nuisance infection that is primarily associated with genitourinary symptoms. It has also been considered a biological marker for high-risk sexual behavior; thus, the detection of *T. vaginalis* would trigger an evaluation for other pathogens. While *T. vaginalis* is associated with sexual risk behavior and other STIs, it is now considered an important independent pathogen. Multiple studies have linked *T. vaginalis* infection to significant and costly adverse health outcomes, such as pelvic inflammatory disease (13, 14), the acquisition of human immunodeficiency virus (5, 10), human immunodeficiency virus shedding (7), and cervical cancer (15, 18, 19).

The majority of clinicians who presently test women for *T. vaginalis* infection rely upon insensitive diagnostic methods, such as wet mount, and asymptomatic women are seldom tested at all. Although wet mount is the standard of care, it is

TABLE 1. Performance of OSOM Trichomonas Rapid Test performed on vaginal swabs, wet mount alone, and OSOM Trichomonas Rapid Test performed on wet-mount saline compared to CRS for the detection of *T. vaginalis*

Test method (no.)	No. true positive	No. false positive	No. false negative	No. true negative	% Sensitivity (95% CI)[a]	% Specificity (95% CI)	PPV[b] (%)	NPV[c] (%)
OSOM swab (437)	85	4	17	331	83.3 (76–91)	98.8 (98–100)	95.5	95.1
Wet mount (449)	75	0	30	344	71.4 (63–80)	NA[d]	100	92.0
OSOM saline[e] (447)	79	5	26	337	75.2 (67–84)	98.5 (97–100)	94.0	92.9

[a] CI, confidence interval.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.
[d] NA, not applicable (set at 100%).
[e] Saline from wet-mount preparation tested with OSOM.

only 60% sensitive compared to culture (17). In many health care settings, the lack of an experienced microscopist precludes accurate detection of *T. vaginalis*. In some settings, the wet mount is transported to the microbiology laboratory and read by technicians after a significant time delay. The sensitivity of wet-mount microscopy for detecting *T. vaginalis* declines substantially with even relatively short time intervals between collection and examination (8). Where wet mount is available, the appropriate *T. vaginalis* test for wet-mount-negative subjects has not been delineated. We have demonstrated that OSOM can be used on saline solution after a traditional wet mount is performed and can identify *T. vaginalis* not detected by wet mount.

In this study, *T. vaginalis* was common (23.4%) in sexually active women presenting with signs and symptoms of vaginitis. Consistent with previous reports (2, 3), the prevalence of *T. vaginalis* did not decrease with age, as is seen with other STDs, such as *C. trachomatis* and *N. gonorrhoeae* infections. Though women over age 30 constituted one-third of our sample, they contributed almost one-half (47%) of all *T. vaginalis* infections. Symptomatic women over age 30 may be a target group for *T. vaginalis* testing.

Since the OSOM rapid test is a point-of-care test, it would enhance contact tracing in a difficult-to-reach population. As a result, the test could have an important impact on individual, as well as societal, consequences of untreated STDs. In addition, this rapid test is projected to cost significantly less than culture and nucleic acid amplification methods and approximately the same as wet mount when cost estimates are based on a technician's time.

The limitations of this study are that the OSOM test was performed on frozen samples in batches in a research setting. However, the manufacturer's data demonstrated that freezing and transport of specimens did not appreciably alter the test characteristics (T. Pisani [Genzyme Diagnostics], personal communication), and they have been used for other assays (12). Our patient population was at high risk for STIs in general, and the results may not be comparable in another setting. Further studies are needed to confirm our findings in other patient groups and in clinical sites to assess user variability.

We demonstrated that the OSOM test is more sensitive than wet mount for detecting *T. vaginalis* in a research setting with expert microscopists. It requires less technical expertise and time than *T. vaginalis* culture. Test performance was not affected by the presence of other pathogens. OSOM can detect *T. vaginalis* in samples that have a lower organism load and that require longer incubation time in culture before being classified as positive. When performed on wet-mount saline, the rapid test detected several culture-positive samples that were wet mount negative and thus may be a useful adjunct test for wet-mount-negative subjects where culture is unavailable.

The new OSOM rapid test is an immunochromatographic capillary flow (dipstick) assay that is simple to perform and objective and can provide results in 10 min, so it may be applied as a point-of-care test. Thus, it will be an important addition to the repertoire of techniques available for *T. vaginalis* detection, especially for facilities without access to a microscope or incubator and in settings where difficult patient follow-up makes point-of-care testing attractive.

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