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Evaluation of Affirm VP Microbial Identification Test for *Gardnerella vaginalis* and *Trichomonas vaginalis*

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A commercial system (Affirm VP Microbial Identification Test; MicroProbe Corp.) for detection of vaginal pathogens was evaluated with 176 consecutive women attending a sexually transmitted disease clinic for genital complaints. Vaginal swab specimens were used for culture of *Gardnerella vaginalis* and *Trichomonas vaginalis*, preparation of a vaginal smear for Gram stain interpretation, and wet mount evaluation. An additional swab was used to evaluate the 30-min nonisotopic oligonucleotide probe test. The automated probe system detected *G. vaginalis* in 69 (95%) of 73 women having $>5 \times 10^5$ CFU of *G. vaginalis* per ml by culture, and 20 (43%) of 47 specimens with $\leq 5 \times 10^5$ CFU of *G. vaginalis* per ml. There were three false positives and four false negatives for the Affirm VP test compared with $>5 \times 10^5$ CFU of *G. vaginalis* per ml. The probe system detected *G. vaginalis* in 57 (90%) of 63 vaginal specimens from women having clue cells on wet mount examination, and in only 3 (3%) of 113 women without clue cells, suggesting that the Affirm probe for *G. vaginalis* could be used as a surrogate for wet mount examination for clue cells. The *T. vaginalis* probe was positive for 12 of 12 specimens positive by wet mount and 12 of 15 specimens positive by culture. There were no false positives and three false negatives for the Affirm VP test compared with culture and/or wet mount for *T. vaginalis*. The Affirm VP Microbial Identification System is a rapid, objective, and automated test for the detection of *T. vaginalis* and clinically significant levels of *G. vaginalis* that is comparable to wet mount examination for clue cells and is superior to wet mount examination for the detection of trichomonads.

Bacterial vaginosis (BV) and trichomoniasis are syndromes associated with abnormal vaginal discharge in adult women and result in as many as 5 to 10 million patient visits annually (17). The diagnosis of these highly prevalent syndromes is based upon the evaluation of clinical symptoms in conjunction with results from microscopic examination of vaginal fluid, vaginal pH, and the KOH amine odor test (1, 4, 5). The clinical symptoms of vaginal infections are nonspecific, subjective, and often subtle. Thus, diagnosis depends on evaluation of several subjective signs, which makes diagnosis difficult, especially in the case of mixed infections. Because women with either syndrome may be asymptomatic, both disorders are often overlooked.

Laboratory tests for the diagnosis of BV have included evaluation of the vaginal flora by Gram stain (16, 21), the detection of the bacterial metabolites by gas-liquid chromatography (10, 20), and a rapid test for the presence of proline aminopeptidase (22). Gram stain diagnosis of BV has been shown to have a sensitivity of 62 to 93% compared with diagnosis by clinical criteria (4, 10, 21). Routine *Gardnerella vaginalis* cultures have little clinical utility since up to half of women without BV harbor this organism (1, 4, 10).

Diagnosis of trichomoniasis is most commonly based upon the demonstration of the motile trichomonads in vaginal fluid by microscopic examination. Past studies have indicated that wet mounts tend to be only 50 to 75% sensitive compared with culture (5, 9, 13). However, culture of *Trichomonas vaginalis* by traditional methods is labor intensive, taking up to 5 days to complete and requiring at least three additional wet mount evaluations by technical staff. A pouch culture method for *T. vaginalis* has been developed, but this technique still requires up to three days of incubation and microscopic evaluation (3). Pap smears have also been used to detect trichomonads from vaginal-cervical smears. However, this method has been shown to have the least sensitivity (34%) of any of the methods (9). Direct immunofluorescence with monoclonal antibody to *T. vaginalis* has been shown to be 86% sensitive (9), and a monoclonal enzyme-linked immunosorbent assay has been demonstrated to be 89% sensitive in research settings (13). Direct immunofluorescent tests for *T. vaginalis* have been commercially available in the past, and may become available in the future. These tests have been categorized as highly complex by the Clinical Laboratory Improvement Act of 1988.

The importance of accurate, reproducible, and rapid laboratory methods to diagnose these syndromes has increased with recent data suggesting an association between these vaginal syndromes and obstetric and gynecologic complications. BV has been associated with an increased risk of histologic chorioamnionitis (7), amniotic fluid infection (18, 24), postcesarean endometritis (24), and postabortal pelvic inflammatory disease (11). Both BV and trichomoniasis have been linked to a 40 to 50% increased risk of preterm delivery and low birthweight (2, 8, 14, 15), as well as to an increased risk of posthysterectomy infections (19).

In this study, we evaluated a rapid, semiautomated, commercial system developed by MicroProbe Corporation, Bothell, Wash. The Affirm VP System is designed to aid in the diagnosis of BV and trichomoniasis in a clinic, physician office, or clinical laboratory which performs moderately complex evaluations as designated by the Clinical Laboratory Improvement Act of 1988. The system uses synthetic oligonucleotide probes for the simultaneous detection of *G. vaginalis* and *T. vaginalis* from a single vaginal swab. The sensitivity, specificity, and predictive values of positive and negative assays for the probes were compared with the

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clinical criteria commonly used for the diagnosis of each syndrome, as well as culture and microscopic results.

MATERIALS AND METHODS

After providing informed consent, 176 women being seen for new genital complaints at the Sexually Transmitted Disease Clinic at Harborview Medical Center in Seattle, Wash., were enrolled in this study. Women who had received an antibiotic or antifungal therapy within the past week or who had douched within 24 h were excluded from this study, since it was thought that these might diminish the quality of the culture results.

Clinical examination was performed by first inserting a sterile, nonlubricated speculum into the vagina to allow visualization of the posterior fornix. The vaginal vault was examined for discharge, and vaginal pH was measured by moistening pH paper, range 4.0 to 7.0 (ColorpHast; MCB Reagents, Gibbstown, N.J.) with vaginal fluid obtained from the lateral vaginal wall. Five vaginal swabs were then obtained from the lateral vaginal walls and the vaginal vault. The vaginal swabs were collected in random order and were randomly selected for the individual test procedures.

One vaginal swab was placed in a sterile tube with 0.2 ml of sterile physiologic saline solution for wet mount evaluation. One drop of the suspension was placed onto a slide with a coverslip and examined microscopically within 15 min by the clinician for motile trichomonads and for the presence of clue cells (vaginal epithelial cells whose peripheral borders were obscured by attached bacteria). A second drop of suspension was mixed with 10% potassium hydroxide and immediately evaluated for the presence of odor (normal, foul, or amine). Of the four remaining swabs, one each was placed into Amies transport media (Medical Media Laboratory, Boring, Oreg.), Diamond's broth media (prepared in-house) (5), and two Affirm collection vials, one for immediate processing and one to evaluate stability of the assay sample either at 4°C or at room temperature.

For recovery of *G. vaginalis*, the Amies swab was inoculated onto a single HBT plate (human blood bilayer agar with Tween 80) (Remel, Fife, Wash.) and incubated under aerobic conditions with 7% CO_2 at 37°C for 48 h (23). All *G. vaginalis* isolates were identified on the basis of hemolysis on human blood agar, Gram stain reaction showing typical gram-negative to gram-variable rods, and negative catalase production (12). To ensure that semiquantitation of cultures was standardized, a 0.5 McFarland standard of *G. vaginalis* was serially diluted 1:10 from 10^{-1} to 10^{-8} and 100 μl of each dilution was then inoculated onto HBT for isolation. One hundred microliters of each dilution was used to inoculate the swab since the average weight of vaginal fluid found to absorb onto a Dacron swab has been determined to be 97 μg (80 to 152 μg) (6). The average range of CFU per quadrant of the agar streak plate was then determined (Table 1).

A vaginal smear was then made by rolling the vaginal swab from the Amies transport media over a glass slide. The heat-fixed slide was then Gram stained with a safranin counterstain and evaluated according to the method described by Nugent et al. (16). Each smear was categorized as normal (*Lactobacillus*-predominant flora), as intermediate, or as indicating BV (decreased *Lactobacillus* morphotypes with increased numbers of small gram-negative rods or gram-variable rods and curved gram-variable rods). The smears were interpreted by microbiologists who had no knowledge of the clinical assessment or of the Affirm test results.

TABLE 1. Comparison of the Affirm VP test for *G. vaginalis* and *T. vaginalis* with culture and wet mount

Target organism and result	No. of isolates with result with Affirm VP test			
	True positive	False positive	True negative	False negative
G. vaginalis				
3 to 4+ (>5 × 10^5 CFU/ml) by culture	69	0	0	4
1 to 2+ (≤5 × 10^5 CFU/ml) by culture	20	0	0	28
Negative by culture	0	3	52	0
T. vaginalis				
Positive by culture only	12	3	152	3
Positive by wet mount only	12	3	155	0
Positive by either	15	0	152	3

The inoculated Diamond's broth for the recovery of *T. vaginalis* was incubated aerobically at 7% CO_2 at 37°C. The broth was examined microscopically for motile trichomonads on days 1, 3, and 5 (5).

The two swabs in Affirm collection vials were processed for hybridization. One swab was processed immediately at room temperature with the addition of 300 μl of lysis solution. The swab was mixed in the solution by twisting the swab with an up-and-down motion for 10 to 15 s. With the swab remaining in the tube, the suspension was incubated for 5 min at 85°C in a heating block. The second vaginal swab was used to evaluate the effect of storage conditions which might occur in a clinical setting on the assay. Approximately half of these duplicate swabs were stored at 4°C for 4 h. The remaining swabs were stored at room temperature for 1 h prior to processing.

After incubation, 450 μl of Affirm buffer solution was added to the lysis suspension. The contents of the swab were expressed along the wall of the tube, and the swab was discarded. The solution was then filtered into the first well of the seven-well processing cassette caddy. Substrate solution for color development of the hybridization was added to the seventh well. The Affirm card containing the embedded beads to which the specific oligonucleotide probes for *G. vaginalis* and *T. vaginalis* (capture beads) were fixed was placed in well 1, and the automated processing was begun. The processor is shown in Fig. 1. Hybridization of the sample nucleic acid with the capture probe occurs in the first well and is followed by hybridization with the biotinylated color development probe in well 2. After a wash in well 3 to remove unbound probe, streptavidin-horseradish peroxidase is bound to the captured nucleic acid in well 4, and this is followed by two more washes in wells 5 and 6 to remove unbound conjugate. In well 7, the indicator substrate is converted to a blue color if bound enzyme conjugate is present on the bead.

After 30 min, the results of the assay were observed visually. A blue color reaction was considered positive for the presence of the target organism (Fig. 2). Each probe card contained a positive control to ensure that the assay was performed correctly and to provide a color standard from which to assess the intensity of the color reaction, as well as a negative control to indicate the occurrence of nonspecific binding during the hybridization. The assay was considered valid only if the positive control turned blue while the negative control remained colorless (Fig. 2).

FIG. 1. Microbial nucleic acids were extracted and placed into the first well of the seven-well caddy of the Affirm processor. The reagent cards are moved automatically through the other wells for hybridization and color development, and the assay is completed in 30 min.

RESULTS

A total of 176 women were enrolled in this study and had vaginal swabs available for the *G. vaginalis* assay. Only 170 vaginal specimens were processed for the detection of *T. vaginalis* since six cultures were nonevaluable because they were mistakenly discarded on day 3. A total of 63 (36%) of the 176 women had clue cells identified by wet mount examination of the vaginal fluid, 61 (35%) were diagnosed with BV on the basis of clinical criteria, 79 (45%) had BV on the basis of Gram stain, and 73 (40%) of the vaginal specimens were found to contain *G. vaginalis* at the level of 3+ to 4+ ($>5 \times 10^5$ CFU/ml) by culture. A total of 12 (7%) of the 170 vaginal specimens were positive for *T. vaginalis* by wet mount examination, and 15 (9%) were positive by culture.

The sensitivity of the Affirm probe for *G. vaginalis* was directly related to the concentration of the organism in vaginal fluid. The probe system detected *G. vaginalis* in 69 (95%) of 73 specimens containing *G. vaginalis* at the level of 3 to 4+ ($>5 \times 10^5$ CFU/ml) by culture and in 20 (43%) of 47 vaginal specimens demonstrated to contain lower quantities of *G. vaginalis* (Table 1). Three specimens which were negative for *G. vaginalis* by culture were positive by the Affirm test. These three false-positive tests were positive for *Gardnerella* morphotypes by direct stain and may therefore reflect lack of culture sensitivity rather than poor probe specificity.

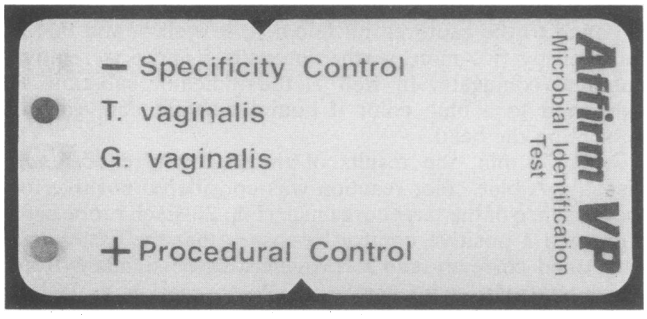

FIG. 2. An Affirm Microbial Identification Test card showing a positive test for *T. vaginalis* and a negative test for *G. vaginalis*. Note the negative specificity control and positive procedural control required for a valid assay.

When compared with the presence of clue cells in the vaginal fluid by microscopic exam, 57 (90%) were positive for *G. vaginalis* by the probe, while only 3 (3%) of the 113 women lacking clue cells were positive by the Affirm VP system. The probe detected *G. vaginalis* in 74 (94%) of the 79 vaginal specimens obtained from women diagnosed with BV by Gram stain, and in 18 (19%) of the 97 women categorized as negative for BV. If culture of *G. vaginalis* at the level of 3+ to 4+ was used as the "gold standard," the Affirm VP system had a sensitivity of 78% (Table 2).

The Affirm probe for *T. vaginalis* detected the organism in all (100%) of the 12 specimens positive by wet mount examination and in 12 (80%) of the 15 vaginal specimens positive by culture (Table 1). The *T. vaginalis* probe also detected the organism in three of six vaginal specimens demonstrated to be wet mount negative but culture positive. The sensitivity and specificity of the probe for *T. vaginalis* were 83% and 100%, respectively, compared with culture and/or wet mount (Table 2).

For each specimen analyzed, the same result was obtained whether the specimen was processed immediately or held at room temperature for 1 h or at 4°C for 4 h prior to processing (data not shown). In addition, the effect of 24 to 72 h of storage on the performance of the Affirm VP test for *Trichomonas* spp. was evaluated in a different group of 81 women (data not shown). The sensitivity and specificity of the assay were 89 and 98%, respectively, compared with culture when the vaginal swab was held at 4°C for up to 72 h in Amies transport media. However, the sensitivity decreased to 67% at 24 h at room temperature, suggesting that refrigeration of the test swab in Amies is necessary if testing is delayed. Port-a-Cul anaerobic transport tubes (BBL,

TABLE 2. Comparison of wet mount, Gram stain, and culture for the diagnosis of BV and *T. vaginalis*

Target organism and result	No. of isolates with result/total no. tested by Affirm VP Probe			
	Sensitivity	Specificity	Predictive value	
			Positive	Negative
G. vaginalis				
Clue cells present	57/63 (90%)	110/113 (97%)	57/60 (95%)	110/116 (95%)
Gram stain BV	74/79 (94%)	79/97 (81%)	74/92 (80%)	79/84 (94%)
Positive by culture (≥3+)	69/73 (95%)	80/103 (78%)	69/92 (75%)	80/84 (95%)
T. vaginalis				
Positive by wet mount only	12/12 (100%)	158/158 (100%)	12/15 (80%)	155/155 (100%)
Positive by culture only	12/15 (80%)	152/155 (98%)	12/15 (80%)	152/155 (98%)
Positive by either	15/18 (83%)	152/152 (100%)	15/15 (100%)	152/155 (98%)

Becton Dickinson Microbiology Systems, Cockeysville, Md.) were also evaluated. The Affirm VP test had lower sensitivity after 24 h at 4°C (72%) and 24 h at room temperature (78%), compared with immediate testing (89%) of a swab transported in a Port-a-Cul tube.

DISCUSSION

The purpose of this investigation was to evaluate the Affirm VP Microbial Identification Test for simultaneous detection of *G. vaginalis* and *T. vaginalis* from a single vaginal swab. This test was developed for use in the physician's office or in the clinical setting as a replacement for the microscopic examination of vaginal fluid for clue cells and trichomonads.

In this study, we found that the oligonucleotide probe for *G. vaginalis* was a very sensitive test for the detection of $>5 \times 10^5$ CFU of the organism per ml and compared well with clue cell detection by wet mount and with diagnosis of BV by Gram stain. Since the Affirm probe for *G. vaginalis* demonstrated a sensitivity of 90% and a specificity of 97% compared with the detection of clue cells by wet mount, the assay could be used as a valid replacement for wet mount examination. However, because the probe detected *G. vaginalis* in 43% of the specimens containing lower quantities of *G. vaginalis* by culture, this test would be most useful in making a diagnosis of BV when used in conjunction with vaginal pH and presence of amine odor. The Affirm test for *G. vaginalis* was positive for 97% of women with a diagnosis of BV based upon clinical criteria and had a specificity of 71%. The only false-positive Affirm tests for *G. vaginalis* occurred with women whose direct smears had *Gardnerella* morphotypes, suggesting that the false-positive results may have been due to the failure of aerobic culture methods to detect obligately anaerobic strains of *G. vaginalis*.

The oligonucleotide probe for *T. vaginalis* was demonstrated to be more sensitive than wet mount (83% versus 67%) compared with true positives (either culture or wet mount positive). Past studies have indicated that examination by wet mount may only be 50 to 75% sensitive compared with culture (5, 9, 13). The probe detected all wet mount-positive specimens, including three which were subsequently culture negative, and there were no false-positive results. However, the probe had three false-negative results, detecting only three of six wet mount-negative, culture-positive specimens. The obvious advantage over culture is that the probe assay takes only 40 min to complete versus 3 to 5 days for standard culture of *T. vaginalis*. In addition, culture is not widely available for this sexually transmitted pathogen.

Several rapid detection techniques have been developed as alternatives to culture and other labor-intensive and cost-ineffective methods. We believe that this automated system provides a useful aid which will help to accurately diagnose these syndromes rapidly in an office-based laboratory.

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ADDENDUM

The product evaluated in the present study was the Affirm VP. However, MicroProbe Corporation markets the product only under the name Affirm VP_{III}, which includes a test for *Candida* species, in addition to *G. vaginalis* and *T. vaginalis*. The utility of the test for direct detection of *Candida* spp. was not evaluated in the present study.

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