

Clinical evaluation of Affirm VPlll in the detection and identification of *Trichomonas vaginalis*, *Gardnerella vaginalis*, and *Candida* species in vaginitis/vaginosis

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Objective: To compare the Affirm VPlll Microbial Identification Test for detection and identification of *Candida* species, *Gardnerella vaginalis* and *Trichomonas vaginalis* to clinical and microscopic criteria commonly used to diagnose vaginitis.

Methods: Women that were symptomatic for vaginitis/vaginosis and asymptomatic women being seen for routine obstetric or gynecological care were included in this study. Women treated with antibiotics or antifungals within one week or women who had douched within 24 hours were excluded. Two vaginal swab specimens were simultaneously obtained from each patient, one swab was placed in sterile physiological saline for immediate microscopic wet mount examination and KOH testing. The other swab was placed in the Affirm collection tube for Affirm VPlll testing based on previously demonstrated methods.

Results: The Affirm assay was significantly more likely to identify *Gardnerella* and *Candida* than wet mount. 190 (45%) were positive for *Gardnerella* by Affirm compared to 58 (14%) by wet mount; 45 (11%) were positive for *Candida* by Affirm compared to 31 (7%) by wet mount; and 30 (7%) were positive for *Trichomonas* by Affirm compared to 23 (5%) by wet mount. Symptomatic women were significantly more likely to be positive by Affirm only (23% vs. 10%), wet mount only (3% vs. 2%) or Affirm and wet mount (15% vs. 1%). Asymptomatic women were significantly more likely to be negative for Affirm and wet mount (43% vs. 5%).

Conclusions: The Affirm VPlll test is a more sensitive diagnostic test for detection and identification of symptomatic vaginitis/vaginosis than conventional clinical examination and wet mount testing.

Key words: VAGINITIS; AFFIRM; EVALUATION

INTRODUCTION

Vaginal discharge is one of the most common women's healthcare problems in the United States¹. Infectious vaginitis is responsible for

between 5 and 10 million clinic visits annually with related healthcare costs of over \$500 million each year². The three leading agents that cause 90% of infectious vaginitis are bacterial vaginosis (BV); fungal infections (yeast); and parasitic

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infections (*Trichomonas vaginalis*). BV is the most common vaginal infection (causing 15–50% of all vaginitis/vaginosis) and *Gardnerella vaginalis* is still considered to be one of the major bacteria causing this infection^{3,4}.

The clinical diagnosis of infectious vaginitis has traditionally relied on tests performed at point of care, such as a physical exam, the appearance of vaginal discharge, a microscopic exam (wet mount), a Potassium hydroxide (KOH) amine odor test, and a pH test of vaginal fluid, as well as laboratory diagnosis via cultures and scored Gram stains⁵. Proper diagnosis and treatment can be elusive if based on clinical symptoms and character of the vaginal discharge alone. This may lead to a lack of relief from symptoms and may increase the costs to the patient from inadequate or improper treatment⁶.

In this study, we evaluated the Affirm VPIII Microbial Identification Test (Becton Dickinson, Sparks, MD, USA) for the detection and identification of *Candida* species, *Gardnerella vaginalis*, and *Trichomonas vaginalis* from vaginal fluid specimens in both symptomatic and asymptomatic patients. The Affirm VPIII test was compared to clinical criteria commonly used to diagnose each syndrome as well as microscopic results.

SUBJECTS AND METHODS

Study design

This study was conducted in Indianapolis, Indiana at two clinical centers: Wishard Health Services (a university-affiliated county hospital) and St. Vincent Hospital (a suburban community hospital). Institutional Review Board approval was obtained and verified by Becton Dickinson prior to patient selection and specimen collection.

Patient selection and specimen collection

Symptomatic women were characterized for inclusion in this study based on clinical findings during examination and/or microscopic examination of the vaginal discharge. Asymptomatic women were characterized for inclusion in this study as those who lacked symptoms of vaginitis/

vaginosis and were being seen for routine obstetric or gynecological exams. Women who had received antibiotic or antifungal therapy within the week or who had douched within 24 hours were excluded. The symptoms, suspected diagnosis, and reason for the visit were recorded and submitted with each specimen.

Clinical examination was performed to include evaluation of vaginal discharge (if applicable). Two vaginal swabs were obtained simultaneously from patients. One swab was placed in sterile physiological saline and immediately examined microscopically for the presence of motile trichomonads, clue cells, and/or yeast or hyphae. A second drop was mixed with 10% KOH and evaluated for the presence of amine odor. All information from both the clinical and microscopic assessments was recorded. The other swab was placed in the Affirm sample collection tube.

Sample testing

The Affirm swab sample was transported to the testing area within 1 hour at room temperature or 4 hours if refrigerated, and stored in the appropriate ambient temperature transport system⁷. The swab was then tested using the Affirm VPIII assay on the BD MicroProbe Processor according to manufacturer's recommendations. The Affirm VPIII test (Becton Dickinson and Company, Sparks, MD, USA) is based on the principles of nucleic acid hybridization and uses two distinct single-stranded probes for each organism, a capture probe and a color development probe. After completion of the test, the results of the assay were visually observed and the results were recorded.

RESULTS

A total of 425 women were enrolled in this study. One hundred and ninety patients (45%) were positive for *Gardnerella* by Affirm, while only 58 (14%) were positive by wet mount (clue cells present). For *Candida*, 45 patients (11%) were positive by the Affirm and 31 (7%) were positive by wet mount. For *Trichomonas*, 30 patients (7%) were positive by Affirm and 23 (5%) were positive by wet mount (Table 1). Patients who

Table 1 Number of specimens positive for each organism for each test

Pathogen (n = 425)	AFFIRM VPIII	Wet Mount	T-statistic	p-value
<i>Trichomonas vaginalis</i>	30 (7%)	23 (5%)	1.2	0.22
<i>Gardnerella vaginalis</i>	190 (45%)	58 (14%)*	10.5	0.00
<i>Candida</i> species	45 (11%)	31 (7%)**	2.0	0.04

*p = 0.001, **p = 0.04

Table 2 Patients positive for *Gardnerella*, *Candida*, and/or *Trichomonas* versus the presence of symptoms for each test system

Result and test system	Symptomatic	Asymptomatic
Positive Affirm only	96 (23%)	42 (10%)**
Positive wet mount only	11 (3%)	8 (2%)*
Positive Affirm and wet mount	63 (15%)	4 (1%)**
Negative Affirm and wet mount	20 (5%)	181 (43%)**

*p < 0.001, ** p < 0.0001

were positive for at least one of these pathogens were further categorized as symptomatic or asymptomatic, as previously defined (Table 2). Symptomatic women were significantly more likely to be positive by Affirm than asymptomatic women (23% vs. 10%) and they were also more likely to be positive by Affirm and wet mount (15% vs. 1%). For asymptomatic women, 43% were negative for Affirm and wet mount, compared to 5% of symptomatic women.

Each patient was ultimately treated for vaginitis/vaginosis based on the results of the wet mount and/or the Affirm test. Patients with clinical signs and symptoms of vaginitis/vaginosis and positive wet mount or Affirm test were treated with appropriate therapy (metronidazole, fluconazole or terconazole). Patients with no symptoms of vaginitis/vaginosis and a positive wet mount or Affirm test were also treated with appropriate therapy. Five per cent of the patients who reported clinical symptoms but exhibited negative wet mount and Affirm test results, were not treated.

DISCUSSION

Sobel reports that three quarters of all adult women experience at least one episode of

candidiasis during their lifetime, with approximately 5% suffering from recurrent infections⁴. Additionally, over 5 million new cases of trichomoniasis are reported each year in the USA alone, and up to 18 million new cases are reported worldwide^{2,8}. Mixed infections are common in up to 25% of patients with infectious vaginitis. Furthermore, up to 50% of vaginal infections are asymptomatic, or diagnosis may be masked by menstruation or prior treatment with over-the-counter creams or douching.

Previous studies have shown that traditional tests for diagnosis and identification of the organism responsible for vaginitis/vaginosis often lack sensitivity or are of little clinical utility. For example, Gram stain diagnosis for BV has been shown to have a sensitivity of 62–93% as compared to clinical diagnosis^{9–11}, and cultures for *G.vaginalis* are of questionable significance since up to 50% of women without BV may be colonized with this organism^{10–12}. For trichomoniasis, microscopic demonstration of motile trichomonads is the most common basis for diagnosis. However, previous studies have reported that wet mounts tend to be significantly less sensitive compared to other methods such as culture and Pap smears^{13,14}. The *Trichomonas* culture, which is considered the gold standard, has improved sensitivity but lacks clinical utility due to an extended turn-around time of up to 5 days.

Clinically, patients with BV or trichomoniasis are at greater risk of becoming HIV positive¹⁵, and may experience increased pregnancy complications such as premature rupture of membranes, preterm delivery and low birth weight². They may also experience an increase in post-surgical gynecological infections^{16–18}.

The Affirm VPIII assay simultaneously detects the presence of clinically significant levels of

Trichomonas, *Gardnerella* and *Candida* from vaginal specimens^{5,7}. This easy to read test has a total hands-on time of approximately 5 minutes, with test results available in less than 1 hour. An extended specimen transport option, Affirm VP8 Ambient Temperature Transport System (ATTS), is also available which extends specimen stability to 72 hours at ambient temperature⁷. Each of these test characteristics is important since it was developed for use in the physician's office as well as the clinical laboratory. Clinicians, particularly in lower prevalence populations, may have difficulty interpreting traditional point of care testing for these pathogens. This phenomenon may account for the vast discrepancy in the detection of *G. vaginalis* from Affirm (45%) and wet mount (14%) in this study.

It is also evident that clinicians rely heavily on diagnostic tests to determine treatment. In this study, 10% of the women positive for *Gardnerella*, *Candida*, and/or *Trichomonas* would not have been treated if clinicians relied on clinical symptoms and wet mount results only. This is especially important for women with BV as it is estimated that up to 50% of these infections are asymptomatic². These statistics are further complicated by the risks involved for both mother and child, if women with BV are pregnant. It has been reported that up to 30% of pregnant women have BV. Additionally, pregnant women with *Trichomonas* and *Gardnerella* infections that are misdiagnosed or not properly treated could experience extreme consequences associated with pre-term and low birth weight babies, which incur greater medical costs and long-term costs in child development. There are new specific therapy options that would rely on a rapid and

accurate differential diagnosis^{18,19}. The need for rapid, accurate and differential diagnosis is further emphasized by the costs of treatment for other risk factors associated with vaginal infections, such as the acquisition and transmission of HIV, other sexually transmitted diseases, as well as the complications associated with pelvic inflammatory disease (PID).

The Affirm VP8 assay is an excellent diagnostic test that will help to rapidly and accurately diagnose vaginitis/vaginosis in both point of care and clinical laboratory settings. Furthermore, we have shown in previous studies with the Affirm test, that clinicians can collect specimens, send them for immediate testing and/or have the transport and test time delayed up to 72 hours with little or no decrease in sensitivity⁷. The Affirm VP8 test is easy to perform and is less cumbersome than traditional point of care tests such as the wet mount test.

The results of this study show that the Affirm VP8 test is more sensitive in symptomatic women than the conventional and more cumbersome point of care wet mount test. As such, we believe that the Affirm's rapid, automated system can offer several advantages in overcoming many diagnostic challenges in certain situations, such as: (1) difficult to diagnose cases in women who frequently douche, are menstruating, or have recently had intercourse; (2) cases with mixed infections that are rarely detected by microscopy; (3) women with recurrent, chronic infections; (4) women with other sexually transmitted diseases that need accurate differentiation and treatment; and (5) pregnant women at high-risk for poor perinatal outcome.

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