Comparison of Affirm VPIII and Papanicolaou Tests in the Detection of Infectious Vaginitis

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Abstract

To compare the Affirm VPIII molecular test (Becton Dickinson, Burlington, NC) with morphologic identification used in routine Papanicolaou (Pap) test screening in the detection and identification of Candida species, Trichomonas vaginalis, and Gardnerella vaginalis, we identified 431 cases with a concomitant Pap test and Affirm VPIII assay performed from the archives of a large academic institution. The study population consisted of women ranging in age from 17 to 79 years (mean and median ages, 33 and 31 years, respectively). With a routine Pap test, 60 patients (13.9%) were found to have bacterial vaginosis, 60 (13.9%) candidiasis, and 3 (0.7%) Trichomonas infection. With the Affirm VPIII assay, 183 (42.5%) patients tested positive for G vaginalis, 70 (16.2%) positive for Candida species, and 10 (2.3%) positive for T vaginalis. The differences were statistically significant. The results demonstrate that our patient population had a high incidence of bacterial vaginosis/Candida vaginitis; however, the Affirm VPIII was a more sensitive diagnostic test for the detection and identification of all 3 organisms compared with the Pap test.

Infectious vaginitis is one of the most common women’s health care problems in the United States.1 The 3 leading microbial agents that are responsible for 90% of infectious vaginitis are the organisms causing bacterial vaginosis (BV), Candida species, and Trichomonas vaginalis.2 Although BV is characterized by a mixed infection of anaerobic bacteria, Gardnerella vaginalis is considered one of the major bacteria causing this infection.3 Optimal treatment relies on the correct identification of these organisms.

Clinical diagnosis of infectious vaginitis relies on physical examination and a number of tests, such as microscopic examination, pH testing of vaginal fluid, and potassium hydroxide (KOH) amine odor test, performed at the point of care. However, such tests are often time-consuming and somewhat subjective. The Papanicolaou (Pap) test has become a routine procedure for women at their annual gynecologic visit because of its success in the prevention of cervical cancer and precursor lesions. In addition to its primary benefit as a cancer screening test, other benefits of the Pap test include the detection of cervicovaginal microorganisms. However, the use of Pap tests to diagnose infectious vaginitis remains controversial.4,5

Recently, a molecular test, Affirm VPIII assay (Becton Dickinson, Burlington, NC) to detect and specifically identify Candida species, G vaginalis, and T vaginalis from vaginal fluid specimens has become commercially available. Preliminary results are promising.6-9 The objective of this study was to compare the Affirm VPIII molecular test with morphologic identification used in routine Pap test screening in the detection and identification of these 3 organisms.
Materials and Methods

Patients who had a Pap test and concomitant Affirm VPIII molecular testing between August 2008 and March 2009 were included in this institutional-approved study. Patients who had Affirm VPIII molecular testing not accompanied by a Pap test were excluded from the study. The specimens were submitted by a dozen community-based gynecologists.

All Pap tests were liquid-based preparations; 20% were ThinPrep (Hologic, Marlboro, MA), and the remaining 80% were SurePath (Becton Dickinson). The Pap tests were not initially identified as part of a study because the cytotechnologists and cytopathologists were blinded to the results of the molecular testing. A diagnosis of BV was made based on the presence of the following 3 findings: a filmy background of small coccobacilli, individual squamous cells coated with a layer of coccobacilli along the cell membranes (so-called clue cells), and conspicuous absence of lactobacilli.

A diagnosis of Candida infection was made by the identification of yeast and pseudohyphae Image 2, whereas a diagnosis of Trichomonas infection was made by the identification of 15- to 30-μm, pear-shaped structures with a centrally located nucleus Image 3.

The samples for the Affirm VPIII molecular test were collected from the posterior fornix and side walls of the vagina using a sterile swab. The swab was then placed into an inactivation medium provided by the vendor (Becton Dickinson). The medium is designed to preserve the nucleic acid of microorganisms during specimen transport at room temperature for up to 72 hours.

The swabs were then tested using the Affirm VPIII assay on the BD MicroProbe Processor (Becton Dickinson) according to the manufacturer’s recommendations. The test is based on the principle of nucleic acid hybridization in which complementary nucleic acid strands align to form specific, double-stranded complexes called hybrids. The system uses 2 distinct single-stranded probes that are complementary to unique genetic sequences of each target organism, a capture probe, and a color-development probe. The capture probe is immobilized on a bead embedded in a Probe Analysis Card (Becton Dickinson). The Probe Analysis Card contains a separate bead for each target organism. The color-development probes are contained in a multiwell reagent cassette.

The assay consists of the following 3 steps: denaturing of the samples to release each target organism’s genetically unique nucleic acids, automated assay processing, and recording of the results after 30 minutes. The assay includes positive and negative controls on each Probe Analysis Card, which are tested simultaneously with each patient sample. After completion of molecular testing, the results of the Affirm VPIII assay were visually observed and recorded Image 4.

Pap tests with results that were discrepant from those of the Affirm VPIII were manually rescreened by a cytotechnologist who was blinded to the results of the original Pap test interpretation and the molecular test results. Statistical analysis was performed by using the χ² test and Fisher exact test. Statistical significance was set at a level of .05 or less.
We used the κ statistic to evaluate chance-corrected agreement between the Pap test and the Affirm VPIII assay. A κ value of 0 indicates that the observed agreement is the same as expected by chance, whereas a κ value of 1 indicates perfect agreement. The values between 0 and 1 represent the various extents of agreement as follows: a value of less than 0.2 indicates poor agreement; 0.21 to 0.6, fair to moderate agreement; 0.61 to 0.8, good agreement; and 0.81 to 1.0, excellent agreement.

Results

The study included 431 cases. The age of the participants ranged from 17 to 79 years with a mean of 33 years. With the Pap test, 60 patients (13.9%) were found to have BV, 60 (13.9%) had candidiasis, and 3 (0.7%) had a Trichomonas infection. With the Affirm VPIII assay, 183 (42.5%) cases tested positive for *G. vaginalis*, 70 (16.2%) were positive for *Candida* species, and 10 (2.3%) were positive for *T. vaginalis*. The differences were statistically significant.

The diagnosis of *Candida* infection by Pap test agreed well with the result of the Affirm VPIII assay (κ = 0.66). There was poor agreement for the diagnosis of BV (κ = 0.32) and *Trichomonas* infection (κ = 0.30).

Coinfection by 2 organisms was noted in 30 cases by using the Affirm VPIII assay; 26 cases tested positive for *Gardnerella* and *Candida*, and the remaining 4 cases were positive for *Gardnerella* and *Trichomonas*. One case was positive for all 3 organisms with the Affirm VPIII assay. Coinfection by 2 or more organisms was noted in only 5 cases based on Pap test evaluation. Of the 5 cases, 4 had BV and candidiasis, and 1 had candidiasis and *Trichomonas* infection. No cases were found to have all 3 organisms using the Pap test.

Of the 431 cases with Pap and Affirm VPIII test results, 154 had discrepant cytologic and molecular test results with regard to the detection and identification of 1 or more of the 3 organisms. Among these 154 discrepant cases, 136 had a positive molecular test result but a negative cytologic result. Among the latter, the Affirm VPIII identified 127 cases of *Gardnerella* infection, 25 of candidiasis, and 7 of trichomoniasis. On rescreening of the Pap slides, BV was identified in 41 cases, candidiasis in 8, and trichomoniasis in 3 that were missed by initial cytologic screening.

There were 18 cases in which the Pap tests reported the presence of organisms, including 13 cases of *Candida*, 4 of BV, and 1 of *Trichomonas*, but the corresponding molecular test results were negative. On rescreening, 8 cases of candidiasis and 2 of BV were confirmed on the Pap tests. The case of trichomoniasis negative by molecular testing was not confirmed by rescreening; the slide demonstrated extensive cytolysis with many naked nuclei that could have been misinterpreted as *Trichomonas*. The Pap tests that were “overinterpreted” for BV demonstrated a filmy background of cocci but lacked evidence of clue cells.
Discussion

Infectious vaginitis is a common medical problem in women. The organisms that cause BV, fungi (candidiasis), and parasites (T vaginalis) are the 3 leading microbial agents that are responsible for 90% of all infectious vaginitis. It has been reported that three quarters of adult women will experience at least 1 episode of vaginal candidiasis during their lifetimes and about half of the women will have 2 or more episodes. Although the incidence of Trichomonas infection has been declining for the past 20 years, more than 7.5 million new cases of Trichomonas infection are reported each year in the United States. BV occurs in up to 30% of the population and is more common in African Americans than Caucasians by a factor of 3. Although affected patients are often concerned by the troubling symptoms, there are also considerable morbidities associated with infectious vaginitis. For example, patients with BV have an enhanced risk of developing postoperative infection after pelvic surgery. BV and trichomoniasis have been associated with adverse pregnancy outcomes such as premature rupture of membranes, preterm delivery, and low birth weight.

The error rate in diagnosing infectious vaginitis using traditional methods is rather high. Based on clinical signs and symptoms and bedside testing, including wet mount microscopy, KOH amine odor test, and a pH test of vaginal fluid, about two thirds of the cases of Candida and BV were misdiagnosed by general practitioners and gynecologists in a cohort of 220 women with vaginal complaints. The authors attributed the error rate to the lack of microscopy-related skills and the subjectivity of some of the diagnostic criteria. Other factors that were noted to have contributed to a misdiagnosis included menstruation, douching, and self-medication. Furthermore, infectious vaginitis can be asymptomatic in as high as 50% of cases. In another study by Lowe et al, the accuracy of the clinical diagnosis of BV, Candida vaginitis, and T vaginalis was compared with the Affirm VPIII DNA probe molecular test for G vaginalis, T vaginalis, and Candida species. Lowe et al found that compared with the DNA probe standard using the Affirm VPIII, clinical diagnosis is 81% to 85% sensitive and 70% to 99% specific for BV, Candida vaginitis, and trichomoniasis. While using traditional standardized clinical diagnostic protocols even under the best of circumstances, the diagnosis and treatment of these common vaginal problems remains challenging.

Some authors advocate the use of Gram stain in the diagnosis of BV. However, Gram stain diagnosis ranged from 60% to 90% compared with the clinical diagnosis of BV. Microbial culture is another technique that can be useful in the diagnosis of vaginitis and vaginosis. Culture for Trichomonas is highly effective and relatively inexpensive. However, it lacks clinical usefulness because it requires a special medium (Diamond), which is not uniformly available, and an extended turnaround time of up to 5 days. Cultures for Gardnerella and Candida species are not recommended because both organisms can be part of the normal vaginal flora without causing any infection.

Because of the success of reducing the incidence and mortality of cervical squamous carcinoma, Pap tests have been widely adopted in the United States and other developed countries. One of the secondary benefits is the detection of microorganisms. As a result, many clinicians have come to rely on the identification of microorganisms by the routine Pap test as part of their patient management. The sensitivity of Pap tests for diagnosing BV varies from 90% to as low as 43%; the wide range of results reflects the use of different morphologic criteria and whether the specimens were obtained from the cervix or the vagina. Similarly, the Pap test lacks sensitivity as a screening test for Trichomonas. Moreover, in low-prevalence populations, Trichomonas found by cytomorphic review and reported by routine Pap testing frequently represented a false-positive result.

There has been an increase in the use of imaging systems for primary screening of Pap tests. For a small portion of scanned slides, no manual review would be performed (if no abnormalities were noted in the fields selected by the system) or only a limited number of fields would be examined (if the system ranks the slides with a low probability of containing abnormalities) depending on the type of imaging system being used. This may result in underdetection of infections. One study observed that the detection rates of Candida (68% vs 77%), Trichomonas (80% vs 85%), and shift in bacterial flora (63% vs 65%) were lower with the AutoPap imaging system (Becton Dickinson) compared with manual screening.

To address these difficulties, a rapid and sensitive tool is needed. The Affirm VPIII assay allows simultaneous detection of the presence of clinically significant levels of Trichomonas, Gardnerella, and Candida from vaginal specimens. The results of our study show that the Affirm VPIII assay demonstrated significantly higher detection rates for all 3 organisms compared with Pap tests. The difference in the detection rate of Gardnerella between the Affirm VPIII assay and the Pap test was the most pronounced. One plausible explanation is that the Pap test is essentially a cervical specimen, while BV is essentially a vaginal condition. Therefore, BV is more likely to be better represented by vaginal samples used in the Affirm VPIII assay. We also observed poor agreement between the Affirm VPIII assay and the Pap test when identifying Trichomonas. As mentioned earlier, in populations like ours with a low prevalence of Trichomonas, a higher incidence of false-negatives and false-positives may be noted in the reporting of Trichomonas by gynecologic cytology staff. Therefore, our findings suggest that the Affirm VPIII assay is more sensitive than the Pap test as a diagnostic tool for the detection and identification of infectious vaginitis.
This argument supported the results of rescreening of the Pap slides with discrepant cytologic and molecular test results. Organisms were identified on rescreening in about one third of the cytologic specimens that were initially reported to be negative for organisms but had positive molecular test results.

Our observation is comparable to findings from studies that compared the Affirm VPIII assay with other methods of detection of these 3 organisms. Based on a cohort of 425 women, Brown et al demonstrated that the Affirm VPIII assay was significantly more sensitive than wet mount in identifying Candida (11% vs 7%) and Gardnerella (45% vs 14%). The Affirm VPIII assay also detected more cases of Trichomonas infection compared with wet mount (7% vs 5%), but the difference was not statistically significant. It has also been reported that the Affirm VPIII assay correlated well with the Gram stain in diagnosing BV and candidiasis.

Because Gardnerella and Candida species can exist as normal flora in 50% of women, a “too sensitive” molecular diagnostic test may yield false-positive results, resulting in unnecessary treatment. To minimize the possibility of false-positive results due to microbial colonization, the detection thresholds of the Affirm VPIII assay were set above the levels of normal flora, thereby ensuring that only clinically relevant cases are detected. According to the manufacturer, the detection thresholds for Gardnerella, Candida, and Trichomonas species are 2 × 10^5 colony-forming units (CFU)/mL, 1 × 10^4 CFU/mL, and 5 × 10^3 CFU/mL, respectively. This may explain some of our “false-negative” molecular test results despite a “positive” cytologic result. Based on personal communications with clinicians, our clients as a whole use the Affirm VPIII molecular test when there is a clinical symptom that is reported by the patient and/or a clinical sign noted during the gynecologic examination. Depending on the provider, in some cases, before ordering the molecular test, other diagnostic tools for vaginosis and vaginitis, such as a wet mount, may be prepared in the office, although much less commonly given the time constraints of office practice and diminishing skill sets. If the bedside point-of-care tests are interpreted as negative, then the Affirm VPIII test is ordered. As a general treatment protocol practiced by our clients, patients with a clinical symptom and/or sign of vaginosis or vaginitis in the setting of a positive result for a given organism(s) (including the Affirm VPIII molecular test result, a wet mount microscopic examination result, and/or a positive Pap test result) are offered treatment for the given organism(s) detected. Using the results of the Affirm VPIII molecular test in addition to the routine Pap test in cases in which there is a clinical suspicion of vaginitis or vaginosis may decrease the likelihood of potential false-positive results and unnecessary treatment.

About 14% of patients with infectious vaginitis have a mixed infection. In our study, the Affirm VPIII assay identified 30 patients (7.0%) with coinfection by 2 or more microorganisms, whereas the Pap test identified only 5 patients (1.2%) with coinfection by 2 microorganisms. The difference was statistically significant (Fisher exact test). In addition, cytologic rescreening detected only 1 additional case of coinfection by 2 pathogens in the “positive for Trichomonas and Gardnerella” category. Our findings suggest that the Affirm VPIII assay is superior to the Pap test in detecting multiple pathogens.

There are other advantages of the Affirm VPIII assay over the Pap test, including the objectivity of the assay, the elimination of the need for special microscopy skills, and the quick turnaround time of less than 2 hours. Also, there is no interference with the assay by over-the-counter medications, lubricants, douching, or menstruation. The specimen transport medium preserves the specimens up to 72 hours from collection to room temperature, thereby facilitating transportation from the physician office to the laboratory.

One of the limitations of our study is that we were not able to estimate the sensitivity and specificity of the Affirm VPIII assay and Pap tests because we did not compare our results with the “gold standards” such as microbial cultures or Gram stain. Other studies reported that the sensitivity and specificity of the Affirm VPIII assay in identifying G vaginalis ranged from 88% to 90% and from 96% to 97%, respectively. For diagnosing Candida species using the Affirm VPIII assay, Petrikkos et al reported a sensitivity of 82% and specificity of 100% when compared with examination using 10% KOH.

We demonstrated that the Affirm VPIII assay using a DNA hybridization technique was more sensitive in identifying G vaginalis, Candida species, and T vaginalis than the Pap test. In addition, mixed infection is more readily diagnosed by the Affirm VPIII assay than the Pap test.

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References


