Comparison of Affirm VpIII and Routine Pap Test in the Detection of Candida Species, Trichomonas Vaginalis, and Gardnerella Vaginalis

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ABSTRACT

Introduction: Although the primary objective of a Pap test is to screen for precancerous lesions, it is clinically helpful to detect the presence of Candida species, Trichomonas vaginalis, and Gardnerella vaginalis, as these organisms are responsible for 90% of vaginitis/vaginosis. Recently, molecular testing using a DNA hybridization technique (Affirm VPIII, Becton, Dickson & Co, Sparks, MD) can be used to detect these 3 organisms from vaginal specimens. The objective of this study is to compare the Affirm VPIII molecular test detection and identification of these 3 organisms to the morphologic identification used in routine Pap test screening.

Materials and Methods: One hundred and ninety-one patients who had a concomitant Pap test and Affirm VPIII assay performed were identified from the archives of a large academic institution. A separate vaginal swab specimen was obtained from each patient after a sample was collected for a Pap test. The swab was then tested using the Affirm VPIII assay. The latter is based on the principles of nucleic acid hybridization, and uses 2 distinct single-stranded probes for each organism; a capture probe and a color development probe.

Results: The study population consisted of women ranging in age from 15 to 86 with a mean and median of 34 and 31, respectively. Table 1 summarizes the frequency of detection of each organism according to each method.

Co-infection by 2 or more organisms was noted in 12 patients using Affirm VPIII, whereas co-infection by 2 or more organisms was only noted in 3 patients based on Pap test evaluation.

Conclusions: Our results demonstrate that our patient population had a high incidence of bacterial vaginosis/candida vaginitis based on either the Affirm VPIII assay or Pap test. However, Affirm VPIII is a more sensitive diagnostic test for detection and identification of all 3 organisms compared to Pap tests. In addition, the Affirm VPIII is superior to Pap tests in identifying patients with mixed infections.

INTRODUCTION

Infectious vaginitis is one of the most common womenís healthcare problems in the United States. The three leading microbial agents that are responsibility for 90% of infectious vaginitis are bacterial vaginosis (BV), candiasis, and Trichomonas vaginalis. Although BV is characterized by a mixed infection of anaerobic bacteria, Gardnerella vaginalis is considered to be one of the major bacteria causing this infection. Optimal treatment relies on correct identification of organisms. Recently, molecular testing has become commercially available for the detection and identification of Candida species, Gardnerella vaginalis, and Trichomonas vaginalis from vaginal fluid specimens. The objective of this study is to compare the Affirm VPIII molecular test (Becton, Dickson & Co, Sparks, MD) for the detection and identification of these 3 organisms to the morphologic identification used in routine Pap test screening.

MATERIALS & METHODS

Women who had a Pap test and concomitant Affirm VPIII molecular testing between August 2008 to March 2009 were included in the study. Women who had Affirm VPIII molecular testing not accompanied by a Pap test were excluded from the study. All Pap tests were liquid based preparations; 20% were ThinPrep (Hologic, Marlboro, MA) and the remaining 80% were Surepath (Becton, Dickson & Co., Bulington, NC). The Pap tests were not initially identified as part of a study. Both the cytotechnologists and cytopathologists were blind to the results of the molecular testing. A diagnosis of bacterial vaginosis was made based on the presence of the following three findings: a filmy background of small coccobacilli, individual squamous cells coated with a layer of coccobacilli along the cell membranes (so called ìclue cellsî), and conspicuous absence of lactobacilli (Bethesda Atlas). A diagnosis of candidiasis infection was made by the identification of yeast and pseudo-hyphae, whereas a diagnosis of trichomonas infection was made by the identification of pear shaped structures, 15-30 microns, with a centrally located nucleus. The swabs were collected and then tested using the Affirm VPIII assay on the BD MicroProbe Processor (Becton, Dickson & Co.) according to the manufacturerís 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 two distinct single-stranded probes for each organism, a capture probe, and a color development. The capture probes are immobilized on a bead embedded in a Probe Analysis Card, which contains a separate bead for each target organism. The color development probes are contained in a multi-well reagent cassette. After completion of the test, the results of the assay are visually observed and the results are recorded. The assay consists of three steps: denaturing of the samples to release the nuclei acids of the target organism, automated assay processing, and recording of the results after 30 minutes. The assay includes a positive control and a negative bead on each probe analysis card, which is tested simultaneously with each patient sample. Statistical analysis was performed using Chi-square test and Fisher exact test. Statistical significance was set at level of 0.05 or smaller. We used the kappa statistic to evaluate chance-corrected agreement between Pap test and Affirm VPIII assay. A kappa value of 0 indicates that the observed agreement is the same as expected by chances whereas a kappa value of 1 indicates prefect agreement. The values between 0 and 1 represent various extent of agreement with a value of less than 0.2 indicating 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

Four hundred and thirty-one women were included in the study. The age of the participants ranged from 17 to 79 years with a mean of 33 years. Table 1 summarizes the results of Pap tests and molecular testing using Affirm VPIII assay. With Pap test, 60 (13.9%) women were found to have BV, 60 (13.9%) candidiasis, and 3 (0.7%) trichomonas infection. With Affirm VPIII assay, 183 (42.5%) women tested positive for Gardnerella vaginalis, 70 (16.2%), tested positive for Candida species, and 10 (2.3%) tested positive for Trichomonas vaginalis. The differences were statistically significant.

The diagnosis of Candida by Pap test agreed well with the result of the Affirm VPIII assay ((= 0.66). There was poor agreement for the diagnosis of BV ((= 0.32) and trichomonas infection ((= 0.30). Co-infection by 2 organisms was noted in 30 patients using the Affirm VPIII assay; 26 patients tested positive for both Gardnerella and Candida, and the remaining 4 patients tested positive for both Gardnerella and Trichomonas. One patient tested positive for all 3 organisms using the Affirm VPIII assay. Co-infection by 2 or more organisms was only noted in 5 patients based on Pap test evaluation. Of those 5 patients, 4 patients were found to have both BV and candidiasis, and 1 patient was found to have both candidiasis and trichomonas infection. No patients were found to have all 3 organisms using the Pap test.

Table 1

	Candida spp.	Trichomonas v.	Gardnerella v.
Pap test	20 (10.5%)	1 (0.5%)	29 (15.2%)
Affirm VPIII	25 (13.1%)	6 (3.1%)	83 (43.5%)
Concordance rate	92.1%	97.4%	71.7%

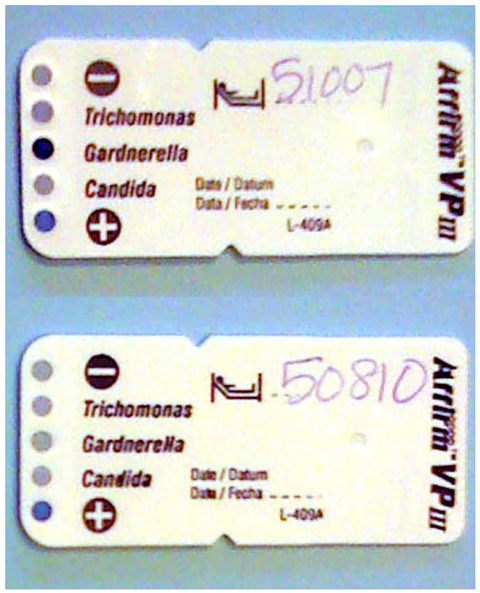

Figure 1.

CONCLUSIONS

- Affirm VPIII assay demonstrated significantly higher detection rates for all 3 organisms when compared to Pap tests; the difference in the detection rate of Gardnerella was the highest.
- Affirm VPIII assay is superior to Pap tests in detecting multiple pathogens.
- Affirm VPIII assay using DNA hybridization is more sensitive than the Pap test as a diagnostic tool for detecting infectious vaginitis and identifying the causative organism(s).
- Other advantages of Affirm VPIII assay over Pap tests are the following:
- Objectivity of the assay.
- No need for special microscopy skills.
- Quick turnaround time of less than 2 hours.
- There is no interference by over-the-counter medications, lubricants, douching, or menstruation.
- Specimen transport media preserves the specimens for up to 72 hours from collection at room temperature.

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