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Comparison of Nucleic Acid Amplification Assays with BD Affirm VP III for Diagnosis of Vaginitis in Symptomatic Women

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A commercially available, nonamplified, nucleic acid probe-based test system (BD Affirm VP III) was compared with nucleic acid amplification (NAA)-based assays for determining the etiology of vaginitis in a cohort of 323 symptomatic women. First, a semiquantitative, multiplexed PCR assay (BV-PCR) and the Affirm VP III Gardnerella vaginalis test were compared with a unified bacterial-vaginosis (BV) reference standard incorporating both Nugent Gram stain scores and Amsel clinical criteria. In the evaluable population of 305 patients, BV-PCR was 96.9% (191/197) sensitive and 92.6% specific (100/108) for BV, while Affirm VP III was 90.1% sensitive (179/197) and 67.6% specific (73/108). Second, a multiplexed PCR assay detecting Candida albicans and Candida glabrata (CAN-PCR) was compared with the Affirm VP III Candida test using a reference standard for vulvovaginal candidiasis (VVC) of yeast culture plus exclusion of alternate vaginitis etiologies. In the population evaluated (n = 102), CAN-PCR was 97.7% sensitive (42/43) and 93.2% specific (55/59) and Affirm VP III was 58.1% sensitive (25/43) and 100% specific (59/59) for VVC. Finally, the results of a commercial NAA test (GenProbe Aptima Trichomonas vaginalis assay; ATV) for T. vaginalis were compared with the Affirm VP III Trichomonas vaginalis test. In the absence of an independent reference standard for trichomonal vaginitis (TV), a positive result in either assay was deemed to represent true infection. In the evaluable cohort of 388 patients, the sensitivity of ATV was 98.1% (53/54) versus 46.3% (25/54) for Affirm VP III. The diagnostic accuracy of the combined NAA-based test construct was approximately 20 to 25% higher than that of the Affirm VP III when modeled in populations with various prevalences of infectious vaginitis.

Vaginitis syndrome, typically characterized by pruritus and vaginal discharge, is one of the most common reasons for women to seek medical attention in the developed world, accounting for 5 to 10 million office visits annually in the United States (1, 2). The majority of cases are infectious in nature, with over 90% of infectious vulvovaginitis being attributable to bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), or trichomonal vaginitis (TV) (3, 4). The clinically nonspecific nature of the syndrome makes use of the laboratory testing essential for the appropriate management of patients; however, historically, this has largely consisted of only rudimentary point-of-care evaluations (pH testing of discharge, microscopic examination, etc.) with limited diagnostic probity (2).

The BD Affirm VP III microbial identification test (Becton, Dickinson, Sparks, MD) is a multianalyte, nucleic acid probe-based assay system designed to enable the identification and differentiation of organisms associated with vaginitis (Gardnerella vaginalis, Candida spp., and Trichomonas vaginalis). Although the Affirm VP III is widely used clinically, only a comparatively small number of studies have been published reporting on the performance characteristics of this test (5–9), and almost all of these only compared the performance of this system against either clinical criteria or clinical criteria supplemented by microscopic examination. Limited data have thus far been presented directly comparing the Affirm VP III with nucleic acid amplification (NAA)-based assays for identifying the primary etiologic agents of vaginitis, despite a growing body of literature documenting the utility of NAA-based tests for diagnosis of this condition (10–13). A recent evaluation of the relative sensitivity of Affirm VP III and a transcription-mediated amplification (TMA)-based test (GenProbe APTIMA Trichomonas vaginalis assay; ATV) for diagnosis of TV demonstrated a 35% increase in sensitivity of T. vaginalis using the amplified methodology (5).

We sought to extend the findings of Andrea and Chapin (5) by independently comparing the results of NAA tests for BV, VVC, and TV with the individual Affirm VP III tests for each of these entities on a cohort of symptomatic women. In addition to comparing the GenProbe ATV with the Affirm VP III T. vaginalis test, a previously described multiplexed PCR test (BV-PCR [10]) was compared with the G. vaginalis component of the Affirm VP III test for determining the BV status of patients, and a novel multiplexed PCR test (CAN-PCR) for Candida albicans and Candida glabrata was compared with the Candida component of the Affirm VP III test.

MATERIALS AND METHODS

Study subjects. The study population was a subset of one described previously (14), consisting of 323 women presenting with clinically documented vaginitis syndrome at either the Sexually Transmitted Diseases Clinic, Jefferson County Department of Public Health (ICDH), Birmingham, AL (n = 288), or the Personal Health Clinic (PHC), University of Alabama-Birmingham, Birmingham, AL (n = 35), between April and October 2011. Approval to conduct this study was obtained from the Western, University of Alabama at Birmingham, and Jefferson Depart-
Sample collection. After informed consent was obtained, a series of vaginal samples was obtained to enable comprehensive evaluation of patients for markers of vaginosis. This sample series consisted of a vaginal swab that was utilized for Gram stain preparation and subsequently placed in an Affirm VPIII transport system (Becton, Dickinson, Sparks, MD), 2 ESwab (Copan Diagnostics Inc., Murrieta, CA) collections for culture and confirmatory Gram stain evaluation, and 2 APTIMA vaginal swab collections (GenProbe Inc., San Diego, CA) for nucleic acid amplification testing.

Conventional diagnostic assessment. Vaginal secretions were collected and evaluated in the respective clinic according to the criteria of Amsel et al. (151). Vaginal samples were also evaluated by quantitative Gram staining at the University of Alabama-Birmingham, to determine the Nugent score (NS [16]), and BV status was defined as a positive NS or as an indeterminate NS plus a positive Amsel score (10). Yeast cultures were performed semiquantitatively by inoculating 50 µl of liquid Amies from Copan ESwab transports onto tryptic soy agar with 5% sheep blood and Sabouraud dextrose brain heart infusion agar with gentamicin. Cultures were incubated with 5% CO2 at 35°C for up to 72 h, plates were examined every 16 to 24 h, and yeast colonies were enumerated. Yeast identification was accomplished by demonstrating germ tube production andSabouraud dextrose brain heart infusion agar with gentamicin. Cultures were incubated with 5% CO2 at 35°C for up to 72 h, plates were examined every 16 to 24 h, and yeast colonies were enumerated. Yeast identification was accomplished by demonstrating germ tube production and subsequently placed in the APTIMA vaginal swab collection system (10). The Affirm VPIII assay was performed and interpreted according to the manufacturer’s instructions on vaginal samples collected and transported in the Affirm VPIII ambient-temperature transport system.

APTIMA Trichomonas vaginalis assay. The ATv assay is a Food and Drug Administration (FDA)-cleared, qualitative NAA test for the detection of rRNA from T. vaginalis. Samples (APTIMA collection system) were analyzed on the fully automated Tigris DTS (Gen-Probe Inc., San Diego, CA) as described in the manufacturer’s instructions.

BV-PCR. BV-PCR was performed as described previously on vaginal samples collected in the APTIMA vaginal swab collection system (10). The BV-PCR assay is a semiquantitative, multiplexed construct containing real-time PCR assays specific for Atopobium vaginae, bacterial vaginosis-associated bacterium 2 (BVAB-2), and Megasphaera type 1. A numerical score is determined for each analyte based on the signal generated in the assay, and the combined score for the 3 analytes is used to assess the presence or absence of BV. A combined score of 0 or 1 indicates the absence of BV, a score of 3 to 6 indicates the presence of BV, and a score of 2 is considered indeterminate for BV.

CAN-PCR. CAN-PCR is a multiplexed, real-time PCR assay utilizing the MultiCode-KTx detection system (Luminex Inc., Madison, WI) that specifically detects C. albicans, C. glabrata, and an internal control (IC) in a single reaction. CAN-PCR master mix contained (in a 25-µl volume) CANFP-Ca/CANRP-Ca/CANFP-Cg/CANRP-Cg primers (see Table 1 for details; 0.2 µM final concentration), Luminex internal control primer set 1 (0.1 µM final concentration, sequence proprietary to Luminex Inc.; 1 IC primer labeled with 6-hexachlorofluorescein [HEX]), ISoluation (1 X [consistent of PCR amplification buffer, MgCl2, and deoxynucleoside triphosphates [dNTPs], including 4-Dimethylaminozo]benzene-4-carboxylic acid [DABCYL]-labeled di-GTP; component of Luminex DNA reaction kit), and Titanium Taq polymerase (0.5 µl; Clontech Laboratories Inc., Mountain View, CA). Following addition of 10 µl of extracted nucleic acid to each reaction (obtained using the MagNA Pure system [Roche Applied Sciences, Indianapolis, IN] as described previously for BV-PCR (10)), amplification reactions were performed on RotorGene Q instruments (Qiagen Inc., Chatsworth, CA) using the following conditions: initial denaturation for 2 min at 95°C, 50 cycles of amplification (95°C for 5 s, 58°C for 10 s, and 72°C for 20 s; fluorescence collected during this step), with postamplification melt analysis (ramp from 60°C to 95°C at 1.0°C per second). Positive samples were identified based on the generation of crossing threshold (CT) values during amplification of <40 cycles and appropriate product peak melting temperature (Tm) signatures in postamplification analysis. Median peak Tm values for the respective amplicons were as follows: C. albicans, 80.4°C; C. glabrata, 73.9°C; and IC, 78.8°C; peak Tm values for amplicons were required to be within 1°C of values generated by the positive control for the appropriate analyte on each individual assay run for the result to be considered positive. Each MagNA Pure extraction tray contained an APTIMA swab collection fluid sample as a negative control. Positive amplification controls (synthetic oligonucleotides) for each Candida sp. were included on each run and required to generate a Ct value within a defined range for the run to be valid. Appropriate amplification of the IC amplicon served to ensure the elimination of PCR inhibitors and recovery of nucleic acid through sample preparation.

RESULTS
Comparison of Affirm VPIII and BV-PCR. The performance characteristics of the Affirm G. vaginalis test and BV-PCR construct compared against the combined NS and Amsel criteria for diagnosis of BV are shown in Table 2. Excluding subjects for whom results were not available for the Affirm VPIII (n = 1) and those whose samples were scored as indeterminate in the BV-PCR assay (n = 17), a total of 305 patients were included in this comparison. Of these patients, 108 (35.5%) were negative for BV and 197 (64.5%) were positive for BV as determined by the previously described combination of NS and Amsel criteria. The Affirm G. vaginalis assay was positive for 35 of the negative patients and negative for 18 of the positive patients, resulting in a sensitivity of 90.1% (179/197; 95% confidence interval [CI], 86.2% to 94.0%), a specificity of 67.6% (73/108; 95% CI, 63.1% to 72.1%), a positive predictive value (PPV) of 83.6% (179/214), and a negative predictive value (NPV) of 80.2% (73/91). The BV-PCR assay was positive for 8 of the negative patients and negative for 6 of the positive patients, resulting in a sensitivity of 96.9% (191/197; 95% CI,
The results of the present study demonstrate that using a combination of the Affirm VPIII assay system are intended to be used in combination to determine the etiology of patients with vaginitis syndrome, we endeavored to compare the overall diagnostic accuracy of this approach with that of the combined results of the NAA tests. To do this analysis, we utilized the performance metrics established in this study for each test and applied them to three distinct patient populations (low, moderate, and high prevalences of infectious vaginitis). High prevalence was represented by a population generally comparable to the one analyzed in the present study (50% BV:35% VVC:15% TV); moderate prevalence by the clinical population served by LabCorp in which NAA testing for vaginitis is ordered (30% BV:25% VVC:10% TV), and low prevalence by a theoretical population (15% BV:10% VVC:5% TV). It was necessary to assume that no patient would have more than one condition simultaneously, to analyze results for the 3 conditions sequentially (BV→VVC→TV), and to assume that the specificity of both Affirm T. vaginalis and ATV testing was 100%. The computation of accuracy was then determined as follows, using the high-prevalence population tested with the Affirm VPIII as an example (Table 3). A theoretical population of 1,000 patients (with an incidence of 50% BV:35% VVC:15% TV) was analyzed using the performance characteristics established experimentally for each test (Table 2). In this example, of the 500 BV-positive patients, 451 would be correctly identified as BV positive using Affirm VPIII and 49 misidentified as BV negative. Of the 500 BV-negative patients, 338 would be correctly identified as BV negative by Affirm VPIII and 162 misidentified as BV positive. Thus, from the Affirm VPIII G. vaginalis test in isolation, 789 (451 BV positive and 338 BV negative) of 1,000 patients would be correctly categorized with respect to BV status. The 338 patients correctly identified as BV negative (and thus potentially VVC or TV positive) were then analyzed using the Affirm VPIII Candida test characteristics. Using an expected incidence of VVC of 35%, 118 patients in this cohort would be expected to be positive for Candida spp., of which 69 would be correctly identified by Affirm VPIII and 49 misidentified. All 220 VVC-negative patients would be correctly identified; thus, after analysis for BV and VVC, 740 of the original 1,000 patients would have been accurately diagnosed with respect to these 2 conditions. The 220 patients correctly identified as negative for BV and VVC were then analyzed using the Affirm VPIII T. vaginalis assay parameters. Using an expected incidence of TV of 15%, 33 patients would be expected to be TV positive, of which 15 would be correctly identified as positive by Affirm VPIII and 18 misidentified as TV negative. In the absence of a determination of specificity for TV, all 187 negative patients were assumed to have been correctly identified as negative for all 3 etiologies. Thus, after complete analysis, a total of 722 (72.2%) of the original 1,000-member cohort would have been accurately stratified with respect to the etiology of their vaginitis symptoms using Affirm VPIII. The same process was repeated for both testing modalities in all 3 populations, and the results of these analyses are shown in Table 3. Using NAA testing generated accurate results on 88.1 to 92.3% of the patients tested, compared with 67.2 to 72.2% for the Affirm VPIII; this difference was significant (0.001) and consistent across all populations analyzed, irrespective of disease incidence.

**DISCUSSION**

The results of the present study demonstrate that using a combination of NAA tests for BV, Candida spp., and T. vaginalis can
result in a significant increase in the accuracy of diagnosis of women presenting with vaginitis syndrome over that which can be accomplished using a nonamplified molecular assay, the Affirm VP III. The predicted diagnostic accuracy of the 3 NAA tests in combination was approximately 20 to 25% higher than that of the Affirm when modeled in populations in which the prevalence of infectious vaginitis ranged from 30% to 100%. The APTIMA vaginal swab collection system, which is the FDA-cleared collection device for the ATV assay, was successfully used for NAA testing for BV and Candida spp., enabling NAA testing for all 3 conditions to be performed from a single collection device. Since this collection device is cleared for use with NAA tests for Chlamydia trachomatis and Neisseria gonorrhoeae, these organisms could also be detected from the same sample, providing adjunctive diagnostic value in the appropriate patient populations (14).

The claimed analytical sensitivities of the Affirm VP III assay vary for the 3 analytes: $5 \times 10^5$ organisms per assay for T. vaginalis, $1 \times 10^4$ CFU for Candida spp., and $2 \times 10^5$ CFU for G. vaginalis (18). The lower relative analytical sensitivity of the G. vaginalis test is intended to improve the specificity of the test for BV, given the high frequency of detection of G. vaginalis in the normal vaginal flora (10, 11, 19). Previous comparisons of the Affirm VP G. vaginalis test with conventional diagnostic tests for BV, primarily the Affirm VP III, have resulted in divergent conclusions regarding the accuracy of this test. Briselden and Hillier (6) in a high-prevalence, symptomatic population reported sensitivity and specificity for the Affirm test versus NS of 99% and 81%, respectively, with intermediate NS samples considered negative for BV. These data were essentially confirmed in a recent study by Crist et al. (8) in a small cohort of symptomatic women in which the Affirm G. vaginalis test was 100% sensitive and 71% specific versus a BV definition of an NS of $>6$. In marked contrast to these studies, Witt et al. (20) found in a low-prevalence population of symptomatic pregnant women a sensitivity of 89.5% and a specificity of 97% for the Affirm G. vaginalis test versus NS irrespective of whether indeterminate NS samples were included as positive for BV or excluded from the analysis (20). The extremely low ratio of samples with NS scores of $>6$ to those with scores of 4 to 6 in the study by Witt et al. (20) suggests that Gram stain scoring in this study may have been extremely aggressive. The findings of the present study are congruent with those reported by Briselden and Hillier (6) and Crist et al. (8), in that we found that the Affirm G. vaginalis test was a relatively sensitive but nonspecific indicator of the presence of BV, as determined by a combination of NS and Amsel criteria, and was markedly inferior in accuracy to the multiplexed BV-PCR test described previously (10). Excluding indeterminate NS samples from the present study improved the specificity of the Affirm test somewhat, from 67.6% (73/108) to 76.4% (68/89); however, a considerable minority of unequivocally BV-negative samples (NS scores of $<4$) generated positive results in the Affirm test. This finding is consistent with observations made previously (10), in which quantitative PCR testing for G. vaginalis was performed on a subset of the current data set. Of the samples tested in that study that were considered negative for BV by NS plus Amsel criteria, 25% contained G. vaginalis concentrations of $>2 \times 10^5$ CFU/ml (10), the purported limit of detection of the Affirm G. vaginalis assay, and 96% (114/119) of samples with G. vaginalis concentrations above this threshold tested positive by Affirm (data not shown). These data support findings from numerous studies that no single marker organism, irrespective of the method utilized in an assay to detect it, accurately identifies patients with BV syndrome (10, 11, 19). Only the use of multiplexed molecular assays that interrogate samples for the presence and quantity of several marker organisms can result in a high level of diagnostic accuracy for this condition (10, 11, 19).

The Affirm Candida test has been subjected to only limited

### TABLE 3 Predicted diagnostic accuracy of Affirm VP III and NAA testing in populations with various prevalences

<table>
<thead>
<tr>
<th>Test system</th>
<th>Population (BV:VVC:TV)</th>
<th>Condition (no. evaluated)$^a$</th>
<th>No. with expected result</th>
<th>No. with predicted result$^b$</th>
<th>Predicted accuracy</th>
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<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>Affirm VP III</td>
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<td>BV (1,000)</td>
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<td>451</td>
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<td>VVC (338)</td>
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<td>220</td>
<td>69</td>
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<td></td>
<td></td>
<td>TV (220)</td>
<td>33</td>
<td>187</td>
<td>15</td>
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<tr>
<td></td>
<td>Moderate incidence (30:25:10)</td>
<td>BV (1,000)</td>
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<td></td>
<td></td>
<td>TV (517)</td>
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<td>NAA assay</td>
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<td>TV (660)</td>
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</table>

$^a$ Indicates number of results that can be analyzed for each test based number of correct results identified in preceding tests using sequential analysis model (BV $\rightarrow$ VVC $\rightarrow$ TV).

$^b$ Results based on sensitivity and specificity parameters determined during the present study. For purposes of analysis, Affirm T. vaginalis test and ATV test were assumed to have 100% specificity. TP, true positive; FN, false negative; TN, true negative; FP, false positive.
comparisons against alternate methodologies, with wet-mount microscopy serving as the primary comparator test (7–9). These evaluations have generally shown the Affirm to detect more positives than wet-mount microscopy (11% versus 7% in the study of Brown et al. [7]), although the absence of a reference standard for what constitutes VVC in these studies precludes determination of clinical sensitivity and specificity parameters. Crist et al. (8) compared Affirm with yeast culture on a small cohort of patients and determined that Affirm was 87% sensitive and 100% specific compared with that particular reference method. A number of studies have also been performed comparing NAA-based approaches to detection of Candida spp. with conventional culture for the diagnosis of VVC (15, 21), with most demonstrating improved sensitivity of NAA versus culture. In the present study, we utilized an objective definition of VVC (symptomatic patients, vaginal swab culture positive for yeast, and no other identified etiology) to compare the performance of Affirm and an NAA test targeting the Candida spp. predominantly responsible for VVC (namely, C. albicans and C. glabrata). The NAA approach was significantly more sensitive at identifying women with VVC than Affirm, 97.7% versus 58.1% ($P < 0.0001$), with the use of an appropriate cutoff value for the PCR assay enabling a high clinical specificity for this test (93.2%) to be sustained. These data illustrate the effectiveness of using a relatively simple PCR construct for VVC, one that detects and differentiates only C. albicans and C. glabrata. Only 2.3% (1/43) of the culture-positive samples were negative in CAN-PCR and contained Candida spp. other than C. albicans or C. glabrata. This finding is consistent with previous studies on the prevalence of different yeast species in unselected cases of VVC (22, 23), infections with Candida spp. other than C. albicans and C. glabrata being largely confined to specific populations and clinical presentations (17).

The absence of an independent reference standard for TV in the present study precluded a determination of clinical specificity and sensitivity for the Affirm VPIII T. vaginalis and ATV tests in the population studied here. The ATV test identified significantly more samples as positive for T. vaginalis than Affirm, resulting in a significantly higher diagnostic yield for this pathogen (16.6% versus 7.8%; $P < 0.005$). These findings are entirely consistent with previous reports on the performance of the ATV assay, either in direct comparison with Affirm VPIII (5) or in comparison with conventional wet-mount examination (12).

Andrea and Chapin (5) observed that of specimens that were falsely negative for T. vaginalis in the Affirm assay, 46.7% were positive in the Affirm G. vaginalis assay, and these authors hypothesized that the cumulative impact of an insensitive test for T. vaginalis used in combination with a relatively nonspecific test for G. vaginalis could cause misdiagnosis and/or less-than-ideal management if an erroneous diagnosis of BV is made in lieu of TV (5). The present study enabled a direct comparison of the performance of the Affirm as a tool for diagnosis of all infectious etiologies of vaginitis and confirmed this hypothesis (Table 3). Irrespective of the overall prevalence of infectious vaginitis in the population tested, use of the Affirm was predicted to result in no greater than 71% correct diagnoses, and thus, approximately 30% of patients for whom this test was used would be at risk of inappropriate management. The use of the combination of NAA tests, with their superior sensitivity for VVC and TV and greater specificity for BV, improved diagnostic accuracy to approximately 90% irrespective of the prevalence of infectious vaginitis in the population.

These data clearly demonstrate the potential for molecular-amplification-based testing to significantly improve the identification of specific etiologies of vaginitis beyond that achievable with a commonly used nonamplified test method. With exciting progress in the development of multiplexed sample-to-answer NAA systems (24), there is the realistic possibility of an NAA testing system comparable to the one described in this report being widely available to clinical laboratories in the relatively near future. The broad adoption of such a testing system would be expected to have a considerable impact on the success rate of managing patients presenting with vaginitis syndrome.

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REFERENCES


