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

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A commercially available, nonamplified, nucleic acid probe-based test system (BD Affirm VPIII) 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 VPIII *Gardnerellavaginalis* 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 VPIII 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 VPIII *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 VPIII *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 VPIII. The diagnostic accuracy of the combined NAA-based test construct was approximately 20 to 25% higher than that of the Affirm VPIII 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 the use of 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 VPIII 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 VPIII 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 VPIII 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 VPIII 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 VPIII tests for each of these entities on a cohort of symptomatic women. In addition to comparing the GenProbe ATV with the Affirm VPIII *T. vaginalis* test, a previously described multiplexed PCR test (BV-PCR [10]) was compared with the *G. vaginalis* component of the Affirm VPIII 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 VPIII 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 (JCDH), 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-

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TABLE 1 Primer sequences used in the CAN-PCR assay for *Candida albicans* and *Candida glabrata*

Target	Primer	Sequence (5′ → 3′)[a]	5′ location[b]	GenBank accession no.
C. albicans	CANFP-Ca	FAM-isodC-TCA.AGT.TGG.GTT.TGC.TTG.AAA.GAC	40	JQ814079
	CANRP-Ca	GGC.AGA.CCT.AAG.CCA.TTG.TCA.AA	78	
C. glabrata	CANFP-Cg	CGA.CTC.GAC.ACT.TTC.TAA.TTA.C	225	JN391275
	CANRP-Cg	FAM-isodC- CCC.CAA.CGA.ACA.AAA.GAA.TAG	273	

[a] FAM, 5-carboxyfluorescein, is used as the reporter dye, coupled to the initial 5′ nucleotide in the primer; isodC, 2′-deoxy-5-methyl-isocytidine, is the initial nucleotide at the 5′ end of the fluorescently labeled primer.
[b] Indicates position within the ribosomal DNA gene locus for the respective organism: internal transcribed spacer 2 for *C. albicans* and internal transcribed spacer 1 for *C. glabrata*.

ment of Public Health Institutional Review Boards. All enrollees were >18 years of age and had not received antibiotics or used vaginal medications for at least 14 days prior to enrollment. The median age of participants was 24 years (range, 19 to 60 years); 93% (300/323) of enrollees were African-American, and 7% (23/323) were white non-Hispanic.

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. (15). 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% CO_2 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 in fetal bovine serum for presumptive identification of *C. albicans*, followed by definitive identification using the Vitek 2 yeast identification card (bioMérieux Inc., Durham, NC). Subjects were considered positive for VVC if a positive culture for *Candida* spp. was obtained and no other etiology identified for the patients' presenting symptoms (17).

Affirm VPIII assay. 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-RTx 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]), ISOlution (1×) {consisting of PCR amplification buffer, $MgCl_2$, and deoxynucleoside triphosphates [dNTPs],including 4-(dimethylaminoazo)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 (C_T) values during amplification of <40 cycles and appropriate product peak melting temperature (T_m) signatures in postamplification analysis. Median peak T_m values for the respective amplicons were as follows: *C. albicans*, 80.4°C; *C. glabrata*, 73.9°C; and IC, 78.8°C; peak T_m 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 oligonucelotides) for each *Candida* sp. were included on each run and required to generate a C_T 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,

TABLE 2 Comparison of results obtained in Affirm VPIII and NAA for diagnosis of vaginitis in symptomatic women

Condition (no. of subjects)	Infection status[a] (no. of subjects)	No. of subjects with result			
		Affirm VPIII		NAA assay[b]	
		Positive	Negative	Positive	Negative
BV ($n = 305$)	Positive ($n = 197$)	179	18	191	6
	Negative ($n = 108$)	35	73	8	100
VVC ($n = 102$)	Positive ($n = 43$)	25	18	42	1
	Negative ($n = 59$)	0	59	4	55
TV ($n = 320$)	Positive ($n = 54$)	25	29	53	1
	Negative ($n = 266$)	0	266	0	266

[a] Infection status determined based on results of conventional diagnostic tests (for BV and VVC); positive results in either Affirm VPIII *T. vaginalis* test or TVA test are considered diagnostic for TV.
[b] NAA assays were BV-PCR, CAN-PCR, and ATV for diagnosis of BV, VVC, and TV respectively.

94.5% to 99.3%), a specificity of 92.6% (100/108; 95% CI, 87.7% to 97.5%), a PPV of 96.0% (191/199), and an NPV of 94.3% (100/106).

Comparison of Affirm VPIII and CAN-PCR. The performance characteristics of the Affirm *Candida* test and the CAN-PCR assay, utilizing a diagnostic definition of VVC as a positive vaginal yeast culture in the absence of an alternate etiology for vaginitis symptoms (i.e., BV or TV), are shown in Table 2. Of the 323 evaluable subjects in the study, 221 (68.4%) were excluded based on a diagnosis of BV (using the NS plus Amsel definition described above) or TV (detection of *T. vaginalis* by either Affirm or ATV). Of the 221 subjects excluded with alternate diagnoses, 168 (76.0%) had BV alone, 16 (7.2%) had TV alone, and 37 (16.8%) had BV and TV. Of the remaining 102 subjects, 43 (42.2%) had a positive yeast culture and thus were deemed to have VVC and 59 (57.8%) had no yeast recovered on culture and thus were considered negative for VVC. The Affirm *Candida* assay was negative for 18 of the positive patients and generated no false-positive results, resulting in a sensitivity of 58.1% (25/43; 95% CI, 43.4% to 72.8%), a specificity of 100% (59/59), a PPV of 100% (25/25), and an NPV of 76.6% (59/77). The CAN-PCR assay was negative for 1 of the positive patients (culture grew *Candida parapsilosis*) and positive for 4 of the 59 negative patients, resulting in a sensitivity of 97.7% (42/43; 95% CI, 93.3% to 100%), a specificity of 93.2% (55/59; 95% CI, 86.8% to 99.6%), a PPV of 91.3% (42/46), and an NPV of 98.2% (55/56).

Comparison of Affirm VPIII and ATV. In the absence of an independent reference standard comparator for TV, only the relative sensitivities of the Affirm VPIII *T. vaginalis* test and the ATV could be assessed in this study, with positive results for either test deemed indicative of infection with *T. vaginalis*. Results of that comparison are shown in Table 2. A total of 3 subjects were excluded from this comparison because of an invalid ATV result, leaving 320 evaluable subjects. A total of 54 patients (16.9%) were identified as positive for *T. vaginalis*, of which 24 were positive in both tests, 1 was positive in the Affirm test only, and 29 were positive only in the ATV assay. The relative sensitivity of the Affirm *T. vaginalis* test was thus 46.3% (25/54; 95% CI, 33.0% to 59.6%), and that for the ATV assay was 98.1% (53/54; 95% CI, 94.5% to 100%).

Estimation of overall diagnostic accuracy. Since the individual components 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 ($P < 0.001$) 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

TABLE 3 Predicted diagnostic accuracy of Affirm VPIII and NAA testing in populations with various prevalences

Test system	Population (BV:VVC:TV)	Condition (no. evaluated)[a]	No. with expected result		No. with predicted result[b]				Predicted accuracy
			Positive	Negative	TP	FN	TN	FP	
Affirm VPIII	High incidence (50:35:15)	BV (1,000)	500	500	451	49	338	162	72.2% (722/1,000)
		VVC (338)	118	220	69	49	220	0	
		TV (220)	33	187	15	18	187	0	
	Moderate incidence (30:25:10)	BV (1,000)	300	700	270	30	473	227	67.5% (675/1,000)
		VVC (473)	118	355	69	49	355	0	
		TV (355)	36	319	17	19	319	0	
	Low incidence (15:10:5)	BV (1,000)	150	850	135	15	575	275	67.2% (672/1,000)
		VVC (575)	58	517	34	24	517	0	
		TV (517)	26	491	12	14	491	0	
NAA assay	High incidence (50:35:15)	BV (1,000)	500	500	485	15	463	37	92.3% (923/1,000)
		VVC (463)	162	301	158	4	281	20	
		TV (281)	42	239	41	1	239	0	
	Moderate incidence (30:25:10)	BV (1,000)	300	700	291	9	644	56	89.7% (897/1,000)
		VVC (644)	161	483	157	4	450	33	
		TV (450)	45	405	44	1	405	0	
	Low incidence (15:10:5)	BV (1,000)	150	850	145	5	787	63	88.1% (881/1,000)
		VVC (787)	79	708	77	2	660	48	
		TV (660)	33	627	32	1	627	0	

[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→VVC→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.

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 VPIII. 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 VPIII assay vary for the 3 analytes: 5×10^3 organisms per assay for *T. vaginalis*, 1×10^4 CFU for *Candida* spp., and 2×10^5 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 NS, 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 94% 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

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 (13, 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-optimal 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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