Reprinted from INFECTIOUS DISEASES IN CLINICAL PRACTICE
Vol. 19 No. 4 July 2011

ORIGINAL ARTICLE

Comparison of the BD Affirm VPIII Test to Primary Care and Clinical Laboratory Methods for the Diagnosis of Bacterial Vaginosis and Yeast Vaginitis

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Background: Diagnosis of bacterial vaginosis (BV) or yeast vaginitis (YV) in the primary care physician's (PCP's) office has relied on patient symptoms, clinical findings during vaginal examination, and analysis of vaginal fluid. These approaches support PCP decisions when selecting patient treatment regimens for these conditions. Alternatively, vaginal specimens may be tested at a clinical or hospital-based laboratory where other diagnostic methods are used including the Nugent Gram stain (NGS), culture, or molecular methods. This study compared these methods and the BD Affirm VPIII (AFF) test to the diagnosis provided by the PCP.

Methods: Eighty-four patients were enrolled at 3 primary care sites. Each site used routine Amsel criteria for BV and wet mount for YV. Three additional vaginal swabs were collected: the first was used to perform a NGS for BV; the second was used for yeast culture; and the third for testing with the AFF, a DNA probe-based method. Primary care physicians diagnosed and treated 36 patients for BV and 30 patients for YV.

Results: Using a NGS score indicative of BV (>6), the sensitivity and specificity of Amsel criteria, AFF, and Nugent GS for BV was 95% and 57%, 100% and 71%, and 50% and 100%, respectively. For YV, the sensitivity and specificity of the wet mount, AFF, and yeast culture was 100% and 11%, 86% and 100%, and 100% and 100%, respectively. Several patients were misdiagnosed and treated inappropriately for BV and YV.

Conclusions: The AFF outperformed and provided a faster result than traditional testing done in the PCP or clinical laboratory setting.

Key Words: bacterial vaginosis, yeast vaginitis, BD Affirm VPIII

(*Infect Dis Clin Pract* 2011;19: 273–275)

Vaginitis is the most common gynecological diagnosis in the primary care setting with more than 50% of women experiencing at least 1 episode of vaginal infection in their lifetime.[1] In the United States, it is estimated that infectious vaginitis accounts for more than 10 million office visits annually at a cost of more than 500 million dollars.[2] The 3 main causes of infectious vaginitis are bacterial vaginosis, vaginal candidiasis, and vaginal trichomonas. Bacterial vaginosis, which typically results from a reduction in lactobacilli in the normal vagina and an overgrowth of *Gardnerella vaginalis* and anaerobes, accounts for 40% to 50% of cases. Vulvovaginal candidiasis accounts for 20% to 25% of cases, and trichomonas vaginitis accounts for 15% to 20% of cases.[3,4] The diagnosis of vaginitis can be difficult because patients may not present with classic symptoms, may be asymptomatic, or may be infected with multiple microorganisms.[5] An incorrect or undiagnosed coinfection can lead to increased morbidity. For example, bacterial vaginosis increases the risk of preterm delivery during pregnancy and has been linked to postabortal pelvic inflammatory disease, postpartum endometritis, and posthysterectomy cuff cellulitis.[6–8]

In the primary care setting, patients presenting with a history or symptoms of vaginitis frequently have a vaginal discharge that can be sampled and analyzed. Standard primary care physician (PCP) office analyses includes a wet mount (WM) preparation using saline, a slide preparation with 10% potassium hydroxide (KOH), a "whiff" test to detect amines, and a litmus test of the pH level of the vaginal discharge. In more difficult cases, the vaginal discharge may be sent to the clinical or hospital-based laboratory for a Nugent Scored Gram stain (NGS) for bacterial vaginosis, a culture for yeast, or microscopy and culture for trichomonas.[5,9]

In an attempt to improve the diagnosis of vaginitis, new diagnostic methods have been developed that detect chemicals, enzymes, antigens, or nucleic acid sequences unique to a particular organism.[9] These tests can be used either in the clinical or hospital-based laboratory or as a point-of-care test in a primary care setting. The purpose of this study was to compare the BD Affirm VPIII Microbial Identification test, a DNA probe–based assay for *G. vaginalis*, *Candida* sp, and *Trichomonas vaginalis*, to primary care and clinical laboratory methods for the diagnosis of bacterial vaginosis and yeast vaginitis.

MATERIALS AND METHODS

Institutional review board approval and patient consent were obtained before specimen collection. There were 84 patients enrolled in the study from 3 primary care sites within a radius of 25 miles of York, Pa (York Women Health Centers: Queen Street, East York, and Shrewsbury). Each site used the following methods for diagnosis of vulvovaginitis, which, for BV, includes: (1) the presence of a homogenous vaginal discharge, (2) vaginal pH greater than 4.5, (3) a positive amine "whiff" test, and (4) microscopic presence of clue cells on a saline WM. Three of 4 of these criteria, when present, are indicative of BV according to Amsel Criteria (AC).[10] For YV, each patient was assessed for (1) the presence of abnormal vaginal discharge, (2) vaginal pH of 4.0 to 4.5, (3) a negative amine test, and (4) microscopic presence of yeast cells or pseudohyphae on a KOH WM. An additional 4 swabs of the vaginal discharge were collected and sent to the Microbiology Laboratory at York Hospital. One specimen was collected using a BBL CultureSwab (BD Diagnostic Systems, Sparks, Md) and tested for BV using the NGS as previously described.[11] The results of the NGS were evaluated using 2 numerical scores: the first score at higher than 6 were those patients with NGS indicative of BV; the second score at higher

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This work was supported in part by a grant from BD Diagnostic Systems, Inc, Sparks, MD.
Disclosure of Competing Interests: Elliot L. Rank and Michelle Althouse are employees of BD Diagnostic Systems, Inc. However, the work was conducted and data analyzed independently at York Hospital by Dr. Crist.

ISSN: 1056-9103

than 3 included patients indicative of BV and those patients with altered vaginal flora. A second specimen was collected with an ESwab transport device that contains a flocked swab in 1 mL of liquid Amies transport medium. Samples (10 and 100 μL) of Amies transport medium were inoculated onto inhibitory mold agar with gentamicin and BBL CHROMagar Candida (BD Diagnostic Systems) for yeast isolation. Yeast cultures were incubated at 35°C for 24 to 72 hours and examined for typical colonial morphology consistent with *Candida* spp. Yeast identification was performed using conventional methods. A third specimen was collected and inoculated into an In Pouch TV *T. vaginalis* test culture medium (Biomed Diagnostics, White City, Ore), which was sent to the laboratory and incubated at 35°C to 37°C. The In Pouch TV was placed on a microscope stage and viewed for the presence of *T. vaginalis*. A fourth specimen was collected with the Affirm Ambient Temperature Transport System for the DNA probe–based Affirm VPIII Test (BD Diagnostic Systems) for BV and YV. The AFF was performed according to the manufacturer's instructions and has been previously described.[12] Patient medical records were reviewed by one of us (R.V.M.) who was blinded to the study results. Data were analyzed by comparing diagnosis and treatment to a consensus of testing methods (CTMs). The CTM is defined as agreement by 2 or more of the methods used for patient evaluation. For example, a positive AC, a positive NGS, and a negative AFF would be considered a positive CTM for BV. Positive results determined in this fashion were then used as the reference by which the efficacy of each individual method was evaluated for statistical purposes.

RESULTS

Review of the 84 medical records for the patients enrolled in this study indicated that there were 30 patients diagnosed and treated for BV by their physicians. There were 24 patients diagnosed and treated for YV. There were 6 patients diagnosed with both BV and YV, and a final 24 patients lacked a diagnosis for either condition. Of the 84 patients, there were 36 patients diagnosed and treated for BV. Twenty-two were positive, and 14 were negative based on a CTM.

Performance characteristics for each method were evaluated using 2 different NGS criteria described in the "Materials and Methods" section. In using an NGS of higher than 6 as the criteria for BV (Table 1), the AC showed excellent sensitivity (95%) but poor specificity (57%). For NGS, the sensitivity was poor (50%), but the specificity was excellent (100%). For AFF, the sensitivity was excellent (100%), but the specificity was lower than the NGS.

TABLE 1. Comparison of Methods in Patients Diagnosed With Bacterial Vaginosis (n = 36)

Method	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
AC	95 (21/22)‡	57 (8/14)	78 (21/27)	89 (8/9)
AFFIRM	100 (22/22)	71 (10/14)	85 (22/26)	100 (10/10)
Nugent GS*	50 (11/22)	100 (14/14)	100 (11/11)	56 (14/25)
AC	92 (22/24)	58 (7/12)	81 (22/27)	78 (7/9)
AFFIRM	96 (23/24)	75 (9/12)	88 (23/26)	90 (9/10)
Nugent GS†	75 (18/24)	100 (12/12)	100 (18/18)	67 (12/18)

*Positive Nugent Score of greater than 6.

†Positive Nugent Score of greater than 3.

‡Percentage (numbers within parentheses show how each percentage was determined).

TABLE 2. Comparison of Methods in Patients Diagnosed With Yeast Vaginitis (n = 30)

Method	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
WM	100 (21/21)*	11 (1/9)	72 (21/29)	100 (1/1)
AFFIRM	86 (18/21)	100 (9/9)	100 (18/18)	75 (9/12)
Yeast culture	100 (21/21)	100 (9/9)	100 (21/21)	100 (9/9)

*Percentage (numbers within parentheses show how each percentage was determined).

Using an NGS score of higher than 3 as the criteria for BV improved the sensitivity of the NGS to 75% without affecting specificity. However, it did not significantly affect the sensitivity or specificity of the AC or AFF observed using the higher NGS score (>6): the sensitivity and specificity for AC were 92% and 58%, respectively, and those for AFF were 96% and 75%, respectively.

There were 30 patients diagnosed and treated for YV. Twenty-one of the 30 were positive, and 9 were negative compared with the reference CTM (Table 2). The WM showed excellent sensitivity (100%) but had the poorest specificity of the methods evaluated (11%). Yeast culture detected all 30 patients (100%) with no false positives. The AFF showed acceptable sensitivity (86%) and excellent specificity (100%).

Trichomonas vaginalis was not identified or isolated in any of the 84 patients who participated in this study; however, 1 patient received a physician diagnosis for TV and was treated empirically.

There were 24 patients with a diagnosis other than BV or YV. Based on the CTM, 5 of these patients were positive for BV, 1 for YV, and 1 for both (Table 3).

DISCUSSION

Primary care physician office–based tests for the clinical diagnosis of BV and YV lack standardization and are not universally used to adequately support a physician's clinical impression for the presence of these diseases in their patient populations.[13] Each of the Amsel criteria is subject to misinterpretation through lack of adequate training, experience, and application. Office-based microscopy is similarly subject to suboptimal use including lack of access to, and proper maintenance of, equipment; inadequate training and ongoing education; and simple misperceptions that microscopy is the only methodology needed in the physician office–based laboratory setting. Ferris et al,[14] in their study findings, categorically state that physicians are not universally skilled in diagnosing vaginosis by microscopic methods, listing a variety of common obstacles that are encountered during the course of routine diagnostic examination.

In a recent study by Lowe et al,[15] clinical diagnosis was 81% to 85% sensitive and 70% to 99% specific for BV, *Candida*, and *Trichomonas* compared with the DNA probe assay. This study was conducted under optimal research conditions that provided clinicians with the time and materials necessary to conduct a thorough and standardized clinical evaluation. In the absence of a controlled research environment, one would assume that the clinical diagnosis would not perform as well.

Our results are consistent with those of Brown et al[12] who found that the Affirm VPIII test was a more sensitive diagnostic test for detection and identification of vaginitis and vaginosis than conventional clinical examination and WM testing.

Our finding of 100% sensitivity via WM KOH for yeast vaginitis in the PCP setting is higher than that previously

TABLE 3. Antimicrobial Therapies Administered to Patients Lacking a Physician Diagnosis of Bacterial Vaginosis or Yeast Vaginitis (n = 24)

Test Results	Antimicrobial Therapy*						No. Patients
	MET	FLUC	NYS	FLUC and NYS	Other	None	
BV	1		2		2		5
Yeast						1	1
BV and yeast	1†						1
Negative for BV and yeast		1	5	2	4	5	17
Total	2	1	7	2	6	6	24

*Treatment: metronidazole, fluconazole, and nystatin.

†Patient diagnosed with *Trichomonas* but negative for *Trichomonas* on WM, culture, and AFFIRM.

FLUC indicates fluconazole; MET, metronidazole; NYS, nystatin.

reported by others.[14] However, the poor specificity of 11% indicates a possible lack of accuracy in interpreting the results on the part of the PCP and raises the possibility of falsely high sensitivity. In addition, with such poor specificity for the WM KOH, 8 of 30 or 27% of the patients in this group were treated with an antifungal agent. This, in addition to the 100% specificity obtained with the AFF and yeast culture, indicates that these patients were unnecessarily treated.

The Nugent GS were read by 2 experienced laboratory medical technologists who performed this procedure on a routine basis. Although the specificity of the NGS was excellent, the sensitivity varied from 50% to 75%, dependent on whether the interpretation was more or less stringent. Our sensitivity findings were lower than those reported by Briselden and Hillier[16] who reported a sensitivity of 94% for the NGS compared with the Affirm VP Probe. Reasons for these differences may be that, in the present study, the NGS was compared with a consensus of methods as a standard, rather than comparison to an individual test. In addition, we cannot exclude the possibility of sample variation because a large number of specimens were required for collection from each of the study participants. Even using a scoring system as part of the NGS evaluation does not rule out all subjectivity in the interpretation of the smears, as compared with a more objective DNA-based probe method.

The 24 patients described in Table 3 illustrate some of the issues with underdiagnosis and overtreatment of this medical condition. Two of the 6 patients diagnosed with BV by the CTM reference were treated appropriately with metronidazole. However, one of the 2 patients was diagnosed by the physician as having *T. vaginalis*, not BV. The physician diagnosis could not be confirmed by any of the 3 approaches, WM, culture, or AFF for this agent. The other patient was not diagnosed with BV but received the antimicrobial agent anyway. An additional 2 patients of the 6 diagnosed by the CTM reference with BV received nystatin. There were 2 patients diagnosed by the CTM reference as having YV; however, neither received antifungal therapy from their physician. Conversely, of the remaining 17 patients, all of whom were negative for BV or YV by the CTM reference methodology, 8 were treated with an antifungal agent.

With our study findings as basis, the BD Affirm VPIII test provides a more objective and accurate diagnosis for BV and YV in either the primary care or clinical laboratory setting.

ACKNOWLEDGMENTS

The authors thank Heather Challenger and Kimberly Young for technical support and Suzanne M. Shultz for reviewing the manuscript.

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