JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1304–1308 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1304–1308.2005

Evaluation of a Point-of-Care Test, BVBlue, and Clinical and Laboratory Criteria for Diagnosis of Bacterial Vaginosis

C. S. Bradshaw,[1,2]* A. N. Morton,[1] S. M. Garland,[3,4] L. B. Horvath,[1,5] I. Kuzevska,[1,5] and C. K. Fairley[1,2]

Melbourne Sexual Health Centre, The Alfred Hospital,[1] School of Population Health,[2] Department of Obstetrics and Gynaecology,[4] and Microbiological Diagnostic Unit,[5] University of Melbourne, and Department of Microbiology and Infectious Diseases, The Royal Women's Hospital,[3] Melbourne, Victoria, Australia

Received 23 September 2004/Returned for modification 7 November 2004/Accepted 20 November 2004

Bacterial vaginosis (BV) remains the most common cause of abnormal vaginal discharge in women of reproductive age and is associated with increased susceptibility to human immunodeficiency virus and sexually transmitted infections and preterm delivery. Present diagnostic methods require access to microscopy and laboratory expertise; however, the majority of women, particularly those in populations with a high prevalence of BV, do not have access to clinical services with on-site microscopy capabilities. We evaluated a point-of-care test for the diagnosis of BV, the BVBlue test, with 288 women attending a sexual health service with symptoms of abnormal vaginal discharge and/or odor. The BVBlue test performed well compared with conventional diagnostic methods for the assessment of women with symptoms suggestive of BV at the bedside and significantly better than other simple tests, such as vaginal pH determination and the amine test, that do not require microscopy. The BVBlue test was sensitive (88%; 95% confidence interval [CI], 81 to 93%) and specific (95%; 95% CI, 91 to 98%) compared to the method of Nugent et al. (R. P. Nugent, M. A. Krohn, and S. L. Hillier, J. Clin. Microbiol. 29:297–301, 1991) and performed well compared with the method of Amsel et al. (R. Amsel, P. A. Totten, C. A. Spiegel, K. C. Chen, D. Eschenbach, and K. K. Holmes, Am. J. Med. 74:14–22, 1983), with a sensitivity of 88% (95% CI, 81 to 93%) and a specificity of 91% (95% CI, 85 to 94%). The BVBlue test is a simple, rapid, and objective test for the diagnosis of BV and has the potential to facilitate prompt diagnosis and appropriate treatment of BV in the absence of microscopy. The majority of women at the greatest risk for the sequelae of BV are not in settings where the conventional diagnostic methods are either practical or possible, and they would greatly benefit from access to rapid and reliable point-of-care tests to improve the diagnosis and management of BV.

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge in women of reproductive age, yet the etiology remains unclear. It is associated with serious sequelae such as preterm delivery (5, 6, 9), spontaneous abortion (6, 14, 18), and increased susceptibility to human immunodeficiency virus (HIV) and sexually transmitted infections (4, 12, 19, 21). Published studies of women in developed nations show considerable variations in the prevalence of BV (5 to 30%), although prevalences in excess of 50% have been reported in studies in sub-Saharan Africa (5, 19). Conventional diagnostic methods for BV require access to microscopy and laboratory expertise; however, the majority of women, particularly those in populations with a high prevalence of BV, do not have access to clinical services with on-site microscopy capabilities. As the clinical signs associated with BV are neither sensitive nor specific (22), misdiagnosis and delays in treatment can place women at further risk of persistent disease, discomfort, and adverse sequelae.

BV is characterized by a disturbance of the normal vaginal flora (8), with a loss of H_2O_2-producing *Lactobacillus* spp. and an increase in the numbers of gram-variable coccobacilli (*Gardnerella vaginalis* and *Bacteroides* spp.), anaerobic organisms (*Mobiluncus* spp., *Fusobacterium* spp., *Prevotella* spp., and *Peptostreptococcus* spp.), and genital mycoplasmas (*Mycoplasma hominis* and *Ureaplasma urealyticum*). Associated with these changes in the vaginal flora are a rise in the vaginal pH and increased levels of production of proteolytic enzymes, organic acids, and volatile amines. Conventional diagnostic methods for BV include the methods of Amsel et al. (1) (the Amsel method) and Nugent et al. (16) (the Nugent method), and although they are widely used, both require microscopy and are unable to provide a simple, objective, and rapid means of diagnosis of BV at the bedside. Several point-of-care tests that do not depend upon microscopy have been developed. These are based on the detection of bacterial amines (23–25) and bacterial sialidase (2), an enzyme produced by anaerobic flora such as *Prevotella* and *Bacteroides* spp. Sialidase promotes adhesion of bacteria to epithelia and also has mucinase activity, which may facilitate invasion of the upper genital tract by BV-associated flora (10, 17, 27). Elevated bacterial sialidase activity has been significantly associated with BV (2) and with preterm birth in women with BV (3, 13). The BVBlue test is a newly developed chromogenic point-of-care test for the diagnosis of BV which is based on detection of increased vaginal fluid sialidase activity ($\geq$7.8 U). To date, only one published study (15) has evaluated its performance and in that study of 57 women, 8 were found to have BV.

Point-of-care tests are not yet in widespread use, and the

* Corresponding author. Mailing address: Melbourne Sexual Health Centre, 580 Swanston St., Carlton, 3053, Victoria, Australia, Phone: 61-3-9341 6253. Fax: 61-3-9347 6757. E-mail: cbradshaw@mshc.org.au.

means of diagnosis of BV is still restricted to conventional methods that require a combination of clinical and laboratory criteria. We performed a study to evaluate the performance of the BVBlue test compared to those of the Nugent and Amsel methods with 288 women presenting with symptoms of abnormal vaginal discharge and/or odor in order to establish the utility of this point-of-care test in settings with a high BV prevalence and without on-site microscopy capabilities.

MATERIALS AND METHODS

Women presenting to Melbourne Sexual Health Centre, Melbourne, Victoria, Australia, with symptoms of abnormal vaginal discharge or odor were eligible for enrollment in the study. Women who were pregnant, HIV infected, postmenopausal, not fluent in English, or menstruating or who had used a lubricant or topical vaginal medication within the previous 72 h were excluded.

Participants completed a questionnaire regarding their symptoms and sexual and behavioral practices and underwent a genital examination. Clinicians performed a speculum examination and recorded the appearance of the vaginal discharge. A swab sample of the vaginal secretion was taken from the lateral wall for vaginal pH determination (Spezialindikator strips [pH 2 to 9]; Merck & Co. Inc., Rahway, N.J.), microscopy (wet preparation and Gram stain), and the amine test. A second vaginal swab was placed in the BVBlue vial (Gryphus Diagnostics, L.L.C., Birmingham, Ala.) containing the chromogenic substrate of bacterial sialidase, and a laboratory timer was started. Two drops of BVBlue developer solution were added at 10 min, and a blue-green color was recorded as a positive result and a yellow color were recorded as a negative result. The BVBlue test was performed at a controlled room temperature (23 to 24°C) in this study and was not incubated, as extensive blinded validation studies by the manufacturer have shown the same visual performance results at temperatures of 22 to 37°C as at incubation at a temperature of 37°C (Stephen Johnson [Gryphus Diagnostics], personal communication).

Evaluation of the vaginal Gram-stained smear, the wet preparation, and the amine test results were performed on site by two laboratory staff trained in both the Nugent and the Amsel methods. The Nugent method is a standardized point scoring system (scores from 0 to 10) based on the presence of three bacterial morphotypes: large gram-positive rods (*Lactobacillus* spp.), small gram-negative or gram-variable coccobacilli (*Gardnerella* and anaerobic spp.), and curved gram-variable rods (*Mobiluncus* spp.) (16). A Nugent score (NS) of 0 to 3 is classified as the presence of normal flora, an NS of 4 to 6 is classified as the presence of an intermediate increase in flora (intermediate flora), and an NS of 7 to 10 is classified as BV. The Amsel method is based on the presence of three of four clinical features: a characteristic homogeneous white adherent vaginal discharge, a vaginal fluid pH >4.5, a positive amine test, and the presence of clue cells (1).

NSs, Amsel scores, and BVBlue test results were all available during the consultation. Women were diagnosed with BV if they had an NS of 7 to 10 or if they were positive for three to four of the Amsel criteria and were treated for 7 days with oral metronidazole or vaginal clindamycin cream if metronidazole was not tolerated. Women underwent screening for *Chlamydia trachomatis* by strand displacement amplification (BD ProbeTec ET CT Amplified DNA Assay; Becton Dickinson & Co., Sparks, Md.), *Neisseria gonorrhoeae* by culture (modified Thayer-Martin medium), and *Trichomonas vaginalis* by culture (modified Diamonds medium).

Patient samples, questionnaires, and clinician checklists were labeled with a unique identifier to ensure confidentiality. This study was approved by the Human Research and Ethics Committee of the Department of Human Services, Melbourne, Victoria, Australia.

Data were entered and stored in Microsoft Access software and analyzed by using SPSS software (version 11.5; SPSS Inc., Chicago, Ill.). Proportions were compared by the chi-square and Fisher's exact tests where appropriate, and 95% confidence intervals (CIs) were calculated. Patients were excluded from the analysis when clinical information or specimens were not available.

RESULTS

Characteristics of the women enrolled. A total of 288 women consented to participate in the study from July 2003 to April 2004. All women reported symptoms of abnormal vaginal discharge or odor at the time of enrollment. The characteristics of the women participating in the study are outlined in Table 1.

TABLE 1. Characteristics of 288 women enrolled in the study

Characteristic[a]	Value (%)
Demographic and behavioral features	
Mean (SD) age (yr)	29 (8)
No. (%) of women who had:	
One or more MSP, last 3 mo	249 (91)
One or more FSP, last 3 mo	25 (9)
Engaged in sex work in last 12 mo	31 (13)
Self-reported past history BV	102 (38)
Laboratory characteristics as no. (%) of women with:	
Yeast infection, by on microscopy	43 (15)
C. trachomatis infection, by DNA assay	7 (3)
N. gonorrhoeae, by culture	2 (1)
T. vaginalis infection, by culture	2 (1)
Three or more Amsel criteria	118 (41)
Nugent score of 7–10	108 (38)
BVBlue test positive	120 (42)

[a] Abbreviations: MSP, male sexual partner; FSP, female sexual partner; percentages are for those with available data who had the characteristic. Note that data were missing for up to 22 patients for behavioral data only.

Fifty percent of the participants had abnormal vaginal flora, with an NS of 4 to 10. BV was diagnosed by the Nugent method in 38% of the women (NSs, 7 to 10), by the Amsel method in 41% of the women, and by the BVBlue test in 42% of the women. Yeasts (buds and/or pseudohyphae), as detected by microscopy, were significantly more common in women with normal and intermediate flora (22.2%) than in women with BV (2.8%), and sexually transmitted infections were uncommon.

Clinical and laboratory findings. All clinical and laboratory findings other than a vaginal discharge typical of BV were uncommon in women with normal flora (NSs, 0 to 3) (Table 2). The BVBlue test result was positive for 88% of women with NSs $\geq$7 but only 5% of women with NSs $\leq$3 ($P < 0.01$). A high proportion (72%) of women with intermediate flora had clue cells, and 54% were diagnosed with BV by the Amsel method and 50% were diagnosed with BV by the BVBlue test.

Performance of BVBlue test compared to those of the Nugent and Amsel methods. A positive BVBlue test result was strongly associated with each of the Amsel criteria, particularly the presence of clue cells, and three or more of the Amstel criteria ($P < 0.01$).

The BVBlue test performed well compared with the performances of the Nugent and Amsel methods (Table 3). It was specific, with a high positive predictive value (PPV) for the presence of both abnormal flora (NSs, 4 to 10) and BV (NSs, 7 to 10). Exclusion of intermediate flora (NSs, 4 to 6) improved the specificity and negative predictive value (NPV) of the BVBlue test compared to the performance of the Nugent method but did not significantly alter the performance of the BVBlue test relative to that of the Amsel method. Only 1 of 288 BVBlue test results was inconclusive, in which there was difficulty in distinguishing between a yellow and a green color change. This occurred in a patient with intermediate flora (NS, 4), a negative amine test result, and a vaginal pH of 4.5 and in whom only two of the Amsel criteria (a classical BV discharge and clue cells) were present.

Comparison of the Amsel criteria and BVBlue test to NSs of 7 to 10. A homogeneous grey-white vaginal discharge was a poor predictor of BV (NSs, 7 to 10) (Table 4). The most reliable indicator of NSs of 7 to 10 was the presence of clue

TABLE 2. Clinical and laboratory findings for the women categorized by NS

Finding	% Women with NS of:			*P* value
	0–3 (n = 144)	4–6 (n = 36)	7–10 (n = 108)	
BVBlue test positive	5	50	88	<0.01[a]
Positive for three or four Amsel criteria	1	54	91	<0.01[b]
Clue cells present	1	72	96	<0.01[b]
Positive amine test	0	34	69	<0.01[b]
pH >4.5	22	74	96	<0.01[a]
Discharge typical of BV	54	69	84	<0.01[a]

[a] Chi-square test.
[b] Fisher exact test.

cells, with a sensitivity of 96% and a specificity of 99%. A positive amine test was highly specific but had a low sensitivity (69%), while a vaginal pH >4.5 was sensitive but was not specific for BV (78%). As these simple tests did not require microscopy and could be performed at the bedside, they were combined with the BVBlue test to determine if the performance of the BVBlue test could be improved. Combination of the BVBlue test result with either a pH >4.5 or a positive amine test result improved either the sensitivity or the specificity of the BVBlue test, but at the expense of the alternative measure. Making a diagnosis of BV if either the BVBlue test result or the amine test result was positive was the best-performing approach, with a high sensitivity and no loss of specificity; however, this improvement was not statistically significant compared to the BVBlue test result alone.

DISCUSSION

The BVBlue test performed well compared with conventional diagnostic methods for the assessment of women with symptoms suggestive of BV at the bedside and performed significantly better than other simple tests, such as vaginal pH determination and the amine test, which do not require microscopy. A reliance on pH alone would lead to the overtreatment of many women, particularly in populations with a low prevalence of BV. A positive amine test result was an excellent predictor of disease, but its low sensitivity means that a considerable proportion of women with BV would be missed. The BVBlue test was more sensitive than the amine test and detection of the presence of the classical homogeneous grey-white discharge and was more specific than elevated vaginal pH (>4.5). Combination of the BVBlue test with the amine test or detection of an elevated vaginal pH did not enhance the performance of the BVBlue test. As has been reported previously (22), clue cells are the most reliable single indicator of BV; however, identification of clue cells requires on-site microscopy capabilities. The BVBlue test is able provide a more objective and more rapid diagnosis of BV at the bedside compared to conventional diagnostic methods. This study was conducted with a population with a high prevalence of BV, had a large sample size, and is only the second study to evaluate the performance of the BVBlue test.

Detection of elevated bacterial sialidase activity has previously been reported to be both sensitive and specific for the diagnosis of BV compared to the results of the Nugent method (sensitivity and specificity, 96 and 96%, respectively) (26) and the Amsel method (sensitivity and specificity, 81 and 94%, respectively) (20). However, it has only recently been developed as a point-of-care test (the BVBlue test). The performance of the BVBlue test has previously been evaluated in a study with 57 women presenting for pelvic examination to a sexually transmitted disease and infectious diseases service, and 8 of these women had BV (15). In that study all samples were incubated at 37°C for 10 min prior to addition of the developer solution, a step which is not practical for a point-of-care test in most settings. Compared to the results of the Nugent method, the sensitivity and specificity of the BVBlue test were 92 and 98%, respectively, and the BVBlue test outperformed the Amsel method, which had a sensitivity of only 50% but a specificity of 100%. In our study the performance of the BVBlue test was similar to that of the Nugent method; however, the BVBlue test did not perform better than the Amsel method for the diagnosis of BV. As the specimens in the previous study (15) were incubated at 37°C for 10 min, it was thought that the higher temperature may have improved the performance of the BVBlue test; however, extensive blinded studies performed by the manufacturer showed the same visual performance results at a temperature range of 22 to 37°C as with incubation at a temperature of 37°C. It is therefore more likely that the high sensitivity and the high specificity of the Amsel method in our study was due to the fact that all participants were examined by sexual health physicians who are experienced with the Amsel method and to the fact that the laboratory criteria were interpreted by on-site laboratory staff who work exclusively in sexual health. It is probable that in the majority of clinical settings without on-site sexual health and laboratory expertise, the Amsel criteria would perform inferiorly and simple point-of-care tests such as the BVBlue test would perform relatively better than clinical and microscopy-based criteria.

A difficulty encountered in many diagnostic studies of BV is

TABLE 3. Sensitivity, specificity, PPV, and NPV of BVBlue test compared to the results of the Amsel and Nugent methods

Comparison method	No. of women	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
Amsel method[a]	285	88 (81–93)	91 (85–94)	87 (80–92)	92 (86–95)
Amsel method[b]	251	92 (85–96)	93 (88–96)	89 (82–94)	95 (90–98)
Nugent method[b]	252	88 (81–93)	95 (91–98)	93 (87–97)	91 (86–95)
Nugent method[c]	287	79 (72–85)	97 (92–99)	94 (89–97)	82 (76–87)

[a] All women were included in this analysis.
[b] Women with intermediate flora (NS of 4 to 6) were excluded from this analysis.
[c] Women with intermediate flora included and an NS of 4 to 10 are considered BV positive.

TABLE 4. Sensitivity, specificity, PPV, and NPV of methods investigated compared to the results of the Nugent method[a]

Criterion	No. of women	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
Clinical and laboratory criteria					
BVBlue test positive	252	88 (81–93)	95 (91–98)	93 (87–97)	91 (86–95)
Vaginal fluid pH >4.5	250	96 (91–99)	78 (71–84)	77 (69–84)	97 (92–90)
Positive amine test	251	69 (60–78)	100 (98–100)	100 (95–100)	81 (75–87)
Typical BV discharge	252	84 (77–90)	46 (38–54)	54 (46–61)	80 (70–87)
Clue cells present	252	96 (91–99)	99 (96–100)	99 (95–100)	97 (93–99)
Positive for three to four Amsel criteria	251	91 (84–96)	99 (96–100)	99 (95–100)	93 (88–97)
Paired criteria					
BVBlue test positive and pH >4.5	250	85 (77–91)	97 (93–99)	96 (90–99)	90 (84–94)
BVBlue test positive or pH >4.5	250	99 (95–100)	78 (70–84)	77 (69–84)	99 (95–100)
BVBlue test positive and amine test positive	251	66 (56–75)	100 (98–100)	100 (95–100)	80 (73–85)
BVBlue test positive or amine test positive	251	92 (85–96)	95 (91–98)	93 (87–97)	94 (89–97)

[a] Women with intermediate flora (NS, 4 to 6) were excluded from the analyses.

the management of women with intermediate flora. The presence of intermediate flora has been shown to increase the risk of adverse obstetric outcomes (6) and acquisition of HIV (21). It is considered an unstable state, and although the clinical condition in many women with intermediate flora does progress to BV, not all women appear to develop BV (7). In our study 12.5% of the women had intermediate flora by the Nugent method, and 54% of this group was classified as having BV by the Amsel method and 50% was classified as having BV by the BVBlue test. Published studies evaluating diagnostic methods in comparison with the Nugent method are not consistent in their classification of this group, and there is considerable variation in whether the presence of an intermediate flora is regarded as positive or negative in analyses. The inclusion of women with intermediate flora consistently reduces the levels of performance of other diagnostic methods compared with that of the Nugent method, and variation in their classification leads to difficulties in comparing test performances between studies. Studies show that both the presence of an intermediate flora and bacterial sialidase activity have been independently associated with adverse obstetric outcomes (3, 6, 13). A potential application for a sialidase-based test, which remains subject to further research, may be to assist clinicians with determining which women with intermediate flora may be at the greatest risk of sequelae.

BV is a common and important infection with significant psychological morbidity and potentially serious and costly sequelae at both the individual and the population levels. It is estimated that the population attributable risk of BV for preterm delivery in the United States is 30%, at a cost of $1 billion per annum (11). BV has consistently been shown to increase susceptibility to HIV transmission, with an attributable risk for antenatal HIV seroconversion of 23% in a study conducted in Malawi (21). Current conventional diagnostic methods have clear limitations in the diagnosis of BV. Point-of-care tests have the potential to facilitate the prompt diagnosis of BV and provision of the appropriate treatment for BV at the primary visit. The development of point-of-care tests that are accurate, simple, rapid, low cost, and stable and that do not require high levels of training for their interpretation is integral to improving the syndromic management algorithms for vaginal discharge that at present perform poorly. The majority of women at the greatest risk of the sequelae of BV are not in settings where conventional diagnostic methods are either practical or possible, and they would greatly benefit from access to rapid and reliable point-of-care tests to improve the diagnosis and management of BV.

ACKNOWLEDGMENTS

We thank Gryphus Diagnostics and Stephen Johnson for the donation of the BVBlue test kits that were used in this study, the staff at Melbourne Sexual Health Centre for their involvement and strong support, Karen Kolosar for expertise in rescoring all the slides in this study, Mary Santoro for assistance, and the women who participated in this project.

This study did not receive financial support from any commercial source.

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