Diagnostic Microbiology and Infectious Disease xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Multicenter study establishing the clinical validity of a nucleic-acid amplification–based assay for the diagnosis of bacterial vaginosis

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ARTICLE INFO

Article history:
Received 16 February 2018
Received in revised form 17 May 2018
Accepted 24 May 2018
Available online xxxx

Keywords:
PCR
Molecular
Test
BV

ABSTRACT

The present study sought to validate the clinical performance of a previously described PCR-based assay for the diagnosis of bacterial vaginosis (BV). A total of 1579 patients were enrolled in 5 locations; samples were classified as BV positive (n=538) or negative (n=1,041) based on an algorithm utilizing quantitative Gram-stain analysis of vaginal discharge and clinical evaluation (Amsel criteria); a next-generation sequencing (NGS) approach to determining diversity of vaginal microbiota was used to resolve discordant results between BV-PCR and Nugent/Amsel. BV-PCR demonstrated a sensitivity of 96.0% (483/503) and a specificity of 90.2% (885/981) when measured against the conventional test standard, with 95 samples (6.0%) being classified as indeterminate. After resolution of discordant results by NGS, including elimination of the PCR indeterminate category, the resolved sensitivity, specificity, and positive and negative predictive values of the BV-PCR assay were 98.7%, 95.9%, 92.9%, and 96.9%, respectively. The results of this study conclusively demonstrate that a relatively simple, 3-biomarker, molecular amplification construct can effectively diagnose BV in symptomatic women. Results generated using this assay were congruent with those obtained using conventional and molecular reference methods.

1. Introduction

Bacterial vaginosis (BV) is reportedly the most common lower genital disorder found in reproductive-age women (Kenyon et al., 2013). This condition is characterized by a shift from a relatively homogeneous microflora dominated by lactobacilli to a highly heterogeneous state containing a complex population of anaerobic and microaerophilic organisms (Ravel et al., 2011; Srinivasan et al., 2012). Symptoms associated with presence of the characteristic BV microflora are production of a whitish-gray discharge with an unpleasant odor, accompanied by itching, burning, or pain (Eckert, 2006). Current therapeutic strategies for BV are limited and relatively ineffective; recurrence of dysbiosis and symptoms of BV occurs in up to 50% of women within 12 months (Bradshaw and Brotman, 2015). Numerous studies have reported associations between BV and clinically significant urogenital tract disorders, including upper genital tract infections, pelvic inflammatory disease, and adverse pregnancy outcomes (Leitich et al., 2003; Peipert et al., 1997; Svare et al., 2006), as well as increasing the risk of acquisition of HIV (Atashili et al., 2008), *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* (Gallo et al., 2012). It is important to note, however, that not all studies have confirmed such associations (Ness et al., 2004) and that the absence of definitive diagnostic criteria for BV continues to hamper attempts to understand the broader consequences of this poorly managed syndrome.

Diagnosis of BV has, until relatively recently, been almost entirely dependent on relatively simple and somewhat limited test methodologies. The first attempts to characterize BV were dependent on using a combination of clinical criteria and simple laboratory tests applied to vaginal samples (Amsel et al., 1983), a constellation of assessments that became known as the "Amsel criteria," of which 3 of 4 (abnormal gray discharge, pH.4.5, positive amine test, and presence of epithelial "clue" cells) are required to be positive to establish the diagnosis. This approach was superseded by the use of an assessment of the extent of microbial diversity of vaginal flora via standardized interpretation of Gram-stained preparations of vaginal samples (Nugent et al., 1991). The so-called "Nugent" score became the de facto gold standard for BV diagnosis (Schwebke et al., 1996; Workowksi and Bolan, 2015), and despite obvious limitations (Srinivasan et al., 2013), results from Nugent and Amsel scoring have remained the generally accepted standard against which novel diagnostic test methods for BV are compared (Cartwright et al., 2012; Gaydos et al., 2017).

The complexity of composition of the BV microflora has become increasingly apparent over the past decade as powerful DNA-based methods for analyzing microbial ecosystems have been applied to analyze vaginal samples from various populations (Fettweis et al., 2014; Fredricks et al., 2005; Gajer et al., 2012; Ravel et al., 2011; Srinivasan et al., 2012). These discoveries have afforded opportunities for

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https://doi.org/10.1016/j.diagmicrobio.2018.05.022
0732-8893/

developing improved methods for BV diagnosis (Cartwright et al., 2012; Fredricks et al., 2007; Gaydos et al., 2017; Shipitsyna et al., 2013; Vitali et al., 2015); one such approach, described by Cartwright et al. (2012), relies on semiquantitative, PCR-based, detection of 3 BV-associated organisms [*Atopobium vaginae*, bacterial vaginosis associated bacterium 2 (BVAB-2), and *Megasphaera*-1]. A clinical evaluation of this construct demonstrated diagnostic accuracy that was comparable to a combination of Nugent Gram-stain score and Amsel result (Cartwright et al., 2012); however, this study was conducted in a single, high–BV risk and prevalence location, with a predominantly African-American population.

The intent of the present study was to confirm the findings reported in Cartwright et al. (2012): to validate in a multicenter study, with a diverse patient enrollment, that the performance characteristics of this test support its use as an accurate and objective method for diagnosing BV in symptomatic women. A secondary goal was to assess whether determining microbiome diversity using next-generation sequencing (NGS) of vaginal swab samples could be used as an independent, unbiased reference method for assessing BV test performance.

2. Materials and methods

2.1. Study locations and subject demographics

Five locations were utilized in the present study (NS-002): 1 academic medical center [Site 001 (Alabama)], 2 primary care facilities [Site 002 (Florida) and Site 004 (Arizona)], and 2 specialist OB/GYN clinics [Site 003 (Florida) and Site 005 (Virginia)]. Based on estimated BV prevalence reported by each site prior to study commencement and assuming approximately equal subject enrollment, a BV positivity rate of 25–35% was projected. Since the sensitivity and specificity of the BV-PCR construct determined previously were 96.7% and 92.2%, respectively (Cartwright et al., 2012), to determine with 95% confidence that these parameters were within 2% of the originally reported values, an enrollment of 1200–1700 subjects was required (Buderer, 1996). A total of 1595 subjects were enrolled between August, 2016, and March, 2017, with 1579 (98.6%) successfully completing all testing. All enrollees were aged between 18 and 50, not pregnant, and presenting for evaluation of clinical symptoms consistent with vaginitis/vaginosis as determined by clinicians at individual sites. Subjects were classified as having a primary diagnosis if they had not been evaluated for vaginitis/vaginosis in the previous 28-day period and had not received antibiotics or used vaginal medications for at least 14 days prior to enrollment. Subjects were classified as having a recurrent diagnosis if they had been evaluated for symptoms consistent with vaginosis/vaginitis in the previous 28 days, received standard-of-care diagnostic evaluation and treatment, and were representing with similar or identical symptoms. Enrollment, prevalence of BV as determined by the conventional test algorithm, and key demographic data are shown in Table 1.

2.2. Sample collection and evaluation

After obtaining informed consent, a series of vaginal samples was obtained to enable comprehensive evaluation of subjects for markers of vaginosis. This sample series consisted of 2 vaginal swab samples collected in liquid Amies transport medium (Copan Diagnostics Inc., Murrieta, CA) that were utilized for Gram stain preparation and yeast culture, 1 vaginal swab sample collected in the Affirm™ VPIII transport system (Becton-Dickinson, Sparks, MD), and 2 APTIMA® vaginal swab collections (GenProbe Inc., San Diego, CA). One of the APTIMA® collections was used for performance of the BV-PCR test, plus additional NAA testing for *Trichomonas vaginalis* and *Candida* spp.; the second was retained for microbiome analysis.

Vaginal discharge was analyzed on each subject at enrollment according to Amsel criteria, with a BV-positive sample being one that had 2 of the following 3 criteria: a pH value of greater than 4.5, a positive "whiff test" ("fishy" odor upon addition of KOH), and the presence of clue cells upon microscopic examination. Assessment of the nature (color, consistency, etc.) of vaginal discharge was not included as an Amsel criterion, the subjective nature of this element of the assessment making it impossible to standardize across study locations. For reference analysis by quantitative Gram stain, duplicate smears were prepared as previously described (Cartwright et al., 2012), and 1 slide was then submitted to a single central laboratory for staining and scoring according to the Nugent criteria. In brief, this involves examining stained smears for specific bacterial morphologies and then generating a numerical Nugent score (from 0 to 10). A score of 0–3 is interpreted as normal or negative for BV; a score of 4–6 as intermediate, meaning there is some evidence of dysbiosis but insufficient to call the sample positive for BV; and a score of 7–10 as abnormal or positive for BV. If the initial slide was deemed to be of insufficient quality for analysis, according to the reference laboratory's established criteria, the second slide was stained and examined. If an accurate result could not be obtained with either slide, the subject was excluded from the study. A combined reference method using Nugent Gram stain plus Amsel was used as the comparator for the BV-PCR assay (Cartwright et al., 2012; Gaydos et al., 2017). A negative or positive Nugent score was considered definitive, samples generating intermediate scores that met the Amsel criteria for

Table 1
Study subject demographic information by sites. BV status determined by conventional testing algorithm (Nugent/Amsel).

	Site 001	Site 002	Site 003	Site 004	Site 005	Total
Enrollment	183	216	584	468	128	1579
Median age	26.7	29.1	29.6	26.4	28.8	28.4
Age range	18.4–47.7	18.2–44.9	18.1–49.3	18.2–41.0	18.0–40.8	18.0–49.3
Race/Ethnicity						
White non-Hispanic	6 (3.3%)	178 (82.4%)	113 (19.3%)	252 (53.8%)	54 (42.2%)	603 (38.2%)
White Hispanic	1 (0.5%)	14 (6.5%)	187 (32.0%)	199 (42.5%)	2 (1.6%)	403 (25.5%)
Asian	1 (0.5%)	1 (0.4%)	4 (0.7%)	7 (1.5%)	6 (4.7%)	19 (1.2%)
African-American	174 (95.2%)	21 (9.7%)	261 (44.7%)	10 (2.1%)	66 (51.5%)	532 (33.7%)
Other	1 (0.5%)	2 (0.9%)	19 (3.3%)	0 (0.0%)	0 (0.0%)	22 (1.3%)
Presentation						
Primary	168 (91.8%)	207 (95.8%)	557 (95.4%)	438 (93.6%)	105 (82.0%)	1475 (93.4%)
Recurrent	15 (8.2%)	9 (4.2%)	27 (4.6%)	30 (6.4%)	23 (18.0%)	104 (6.6%)
BV status[a]						
Positive	136	59	208	75	60	538
Negative	47	157	376	393	68	1041
BV prevalence	74.3%	27.3%	35.6%	16.0%	50.8%	34.1%

Please cite this article as: Cartwright CP, et al, Multicenter study establishing the clinical validity of a nucleic-acid amplification–based assay for the diagnosis of bacterial va..., Diagn Microbiol Infect Dis (2018), https://doi.org/10.1016/j.diagmicrobio.2018.05.022