

## Bacterial Vaginosis by NAA

### Background

Bacterial vaginosis (BV) is an infection marked by an overgrowth of anaerobic organisms and a lack of hydrogen peroxide-producing lactobacilli bacteria.<sup>1</sup> It causes about 22% to 50% of vaginitis symptoms<sup>1</sup> and has been linked to preterm labor, pelvic inflammatory disease, and the acquisition of HIV.<sup>2</sup>

### Diagnostic Tools for BV

Traditionally, a clinical diagnosis of BV requires that three out of four Amsel criteria (abnormal gray discharge, pH > 4.5, positive amine test, and >20% epithelial cells being clue cells) be positive.<sup>1</sup> Many research studies use the quantitative Nugent Gram stain test to diagnose BV, where test scores of 0-3 are deemed normal (non-BV), 4-6 intermediate, and 7-10 positive for BV.<sup>3</sup> However; the Nugent Gram stain tends to be subjective, laborious, and somewhat impractical for routine clinical use. Cultures of vaginal samples have not proven useful for diagnosing BV because (1) many BV-associated organisms, such as *Gardnerella vaginalis*, *Mycoplasma hominis*, *Bacteroides* species, and others, can also be normal vaginal flora<sup>1</sup>, and (2) a number of BV indicator organisms cannot be recovered in culture.<sup>2</sup>

The use of a variety of DNA-based testing methods, such as broad-range and quantitative PCR, has identified novel bacteria associated with BV while also providing more objective, quantitative measures of bacterial presence. It has also enabled a greater understanding of the complexity of microflora alterations underlying BV and provided more probative tools for developing improved diagnostic tests.

### LabCorp Clinical Trial

In 2011, LabCorp conducted a clinical trial of 396 participants with the goal of developing a clinically validated test for BV based on nucleic acid amplification and quantification of key indicator organisms. The trial was conducted in collaboration with Dr Jane Schwebke of the University of Alabama at Birmingham. For each patient enrolled in the study, BV status was ascertained by clinical evaluation against the Amsel criteria and by performing a Nugent Gram stain on a vaginal swab sample. Subjects were deemed positive for BV if a Nugent Gram stain score of 7-10 was recorded or if a Nugent score of 4-6 was recorded and subjects met the Amsel clinical criteria for BV. Concomitantly collected vaginal swab samples were independently tested by quantitative PCR for the presence of 5 potential markers of BV. The results of 169 samples tested in this manner were subjected to multivariate statistical analysis to create the optimal diagnostic framework from which LabCorp's Bacterial Vaginosis by Nucleic Acid Amplification (NAA) test was developed, with the final BV by NAA assay being validated by testing all 396 samples collected during the trial.

### Bacterial Vaginosis by NAA Assay Design and Performance

LabCorp's BV by NAA test utilizes quantitative PCR analysis of the 3 most predictive marker organisms from our clinical trial (*Atopobium vaginae*, Bacterial vaginosis-associated bacterium (BVAB)-2, and *Megasphaera*-1). The individual marker organisms are scored according to the following table:

	Organism Concentration (log <sub>10</sub> DNA copies/mL)		
<i>Atopobium vaginae</i>	≤ 5.5	5.5 - 7.0	> 7.0
BVAB-2	≤ 4.5	4.5 - 6.0	> 6.0
<i>Megasphaera</i> -1	≤ 6.0	n/a	> 6.0
	<b>Low = Score of 0</b>	<b>Moderate = Score of 1</b>	<b>High = Score of 2</b>

The sum of each organism's score equals the total score. Samples with a total score of 0-1 are considered negative for BV, samples with a score of 3-6 are positive for BV, and samples with a score of 2 are indeterminate for BV.

Total Score	Interpretation
3 - 6	Positive - indicative of bacterial vaginosis.
0 - 1	Negative - not indicative of bacterial vaginosis.
2	Indeterminate - unable to determine BV status. Additional clinical and laboratory data should be evaluated to establish a diagnosis.

The performance characteristics of this test versus the standard BV diagnosis used in the LabCorp BV trial (Nugent Gram stain score and Amsel clinical diagnosis) are shown in the following table.

Trial Standard	PCR Positive	PCR Negative	Indeterminate
Positive (n=219)	202	8	9
Negative (n=177)	13	152	12

Sensitivity = 96.2%

Specificity = 92.1%

Positive Predictive Value = 94%

Negative Predictive Value = 95%

Indeterminate rate = 5.3%

LabCorp performed PCR testing for *Gardnerella vaginalis* and *Lactobacillus crispatus* during the trial. Both of these organisms were excluded from the final assay. While *G vaginalis* is well known to be associated with BV, the quantity of *G vaginalis*, as measured by PCR, was less predictive than the 3 markers included in the final assay. Apart from the trial, *G vaginalis* has other limitations. A recent study found the presence of *G vaginalis* in 70% of women without BV<sup>4</sup>, demonstrating its limitations as a single-marker organism for BV.<sup>2,5</sup>

Additionally, LabCorp also did not include *Lactobacillus crispatus* in the final assay. Its inclusion did not improve the positive predictive value of the assay. Furthermore, it is documented that the significance of hydrogen peroxide-producing *Lactobacillus* species to the overall vaginal microflora of healthy women differs considerably based on ethnic background<sup>6</sup>, demonstrating a significant possible confounder if included in the assay.

Test Name	Test Number	Components
<b>Bacterial Vaginosis by NAA</b>	<b>180060</b>	<i>Atopobium vaginae</i> , BVAB-2, <i>Megasphaera-1</i>

Relevant Assays		
<b>NuSwab Vaginitis (VG)</b>	<b>180039</b>	Bacterial vaginosis by NAA, <i>C albicans</i> , <i>C glabrata</i> , <i>Trichomonas vaginalis</i>
<b>NuSwab Vaginitis Plus (VG+)</b>	<b>180021</b>	Bacterial vaginosis by NAA, <i>C albicans</i> , <i>C glabrata</i> , <i>Trichomonas vaginalis</i> , <i>Chlamydia</i> , <i>Gonorrhea</i>

Visit the online Test Menu at [www.LabCorp.com](http://www.LabCorp.com) for full test information, including CPT codes and specimen collection requirements.

LabCorp's policy is to provide physicians, in each instance, with the flexibility to choose appropriate tests to assure that the convenience of ordering test combinations/profiles does not prevent physicians who wish to order a test combination/profile from making deliberate informed decisions regarding which tests are medically necessary. All the tests offered in test combinations/profiles may be ordered individually using the LabCorp test request form.

## References

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