Bacterial Vaginosis: Complications and Diagnostic Options
Moderator

Steve Halasey
Chief Editor
Clinical Lab Products
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Speaker

Donna Mayne, BS, (MT)ASCP, (CLS)NCA

Microbiology/Serology/Molecular Diagnostics Laboratory Manager
Sacred Heart Health System
Bacterial Vaginosis:
Complications and Diagnostic Options

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Microbiology/Serology/Molecular Diagnostics Laboratory Manager
Sacred Heart Health System
Click here to learn about Sacred Heart’s history

The Best Care Comes from the Heart
Speaker Disclosures

The following information represents the results of research into testing options for my laboratory.

I have received a speaker honorarium from Sekisui.
Objectives

Define bacterial vaginosis
Examine causes, symptoms, and treatment
Discuss complications
Review diagnostic options
Bacterial Vaginosis Causes, Symptoms, and Treatment
Bacterial Vaginosis Defined

Bacterial vaginosis: BV, vaginal bacteriosis, *Gardnerella vaginitis*, nonspecific vaginitis

Vaginitis versus vaginosis

- “Vaginitis” = vaginal inflammation
  - Candidiasis
  - Trichomoniasis
  - Bacterial vaginosis
- “Vaginosis” = vaginal disease
Bacterial Vaginosis Defined

Imbalance of vaginal flora

Most common vaginal infection in women aged 15 to 44

Prevalence estimated at 21 million in US alone
Bacterial Vaginosis Defined

CDC doesn’t consider BV a sexually transmitted infection

Non-white women have higher prevalence rates than white women

- 51% African-American
- 32% Mexican-American
- 23% White

Male sex partners of women with BV do not need treatment
Symptoms of bacterial vaginosis result from:

- Reduced quantities of *Lactobacillus* sp.
- Increased quantities of *Gardnerella*, *Bacteroides*, *Mobiluncus*, and *Atopobium*
- Increased vaginal fluid pH

Causes of this “imbalanced flora” are unknown.
Bacterial Vaginosis Symptoms

50–80% asymptomatic
Increased vaginal discharge
Mild vaginal itching
Foul odor
Bacterial Vaginosis Treatment

Recommended treatment regimens include one of the following

- Clindamycin cream: 2% one applicator intravaginally, once a day for 7 days
- Metronidazole: 500 mg orally twice a day for 7 days
- Metronidazole gel: 0.75% one applicator intravaginally, once a day for 5 days
Bacterial Vaginosis Treatment

Alternative treatment regimens include one of the following

- Tinidazole: 2 g orally, once a day for 2 days
- Tinidazole: 1 g orally, once a day for 5 days
- Clindamycin: 300 mg orally, twice a day for 7 days
- Clindamycin ovules: 100 mg intravaginally, once a day for 3 days
Bacterial Vaginosis Treatment

Special considerations related to treatment options include the following

• Alcohol must be avoided when taking metronidazole or tinidazole to avoid serious side effects
• Oils in some vaginal antibiotic creams can degrade latex condoms
• Oral metronidazole and oral clindamycin prevent pregnancy complications
Bacterial Vaginosis Complications

Pregnancy-associated complications

- Preterm labor
- Miscarriage
- Premature rupture of membranes
- Postpartum endometritis
Bacterial Vaginosis Complications

Additional pregnancy-associated considerations

• Appropriate treatment has been shown to reduce risk of preterm labor

• Should BV screening be routinely performed for pregnant women (as with GBS)?
Bacterial Vaginosis Complications

Other complications

- Increased risk for sexually transmitted diseases
  - HIV
  - HSV
  - Chlamydia
  - Gonorrhoea
- Lower success with in vitro fertilization
- Associated with tubal factor infertility
- Pelvic inflammatory disease (PID)
Audience Poll

How many tests for bacterial vaginosis did your laboratory perform last month?

- 0–100
- 101–500
- 501–1000
- >1000
Audience Poll

How many tests for bacterial vaginosis did your laboratory perform last month?

![Bar chart showing the distribution of BV tests performed by respondents.]

- 0-100 tests: 70.3% of respondents
- 101-500 tests: 14.9% of respondents
- 501-1000 tests: 9.5% of respondents
- >1000 tests: 5.4% of respondents

N = 74
Lactobacillus versus Gardnerella

Normal vaginal Gram stain
*Lactobacillus* morphotypes

Abnormal vaginal Gram stain
*Gardnerella* morphotypes
Clue Cells

Squamous epithelial cells coated with bacteria (small GNR/coccobacilli)
Previously considered 4+ growth of *Gardnerella* in vaginal culture positive for bacterial vaginosis

- Gram variable coccobacillus/rods
- Facultative anaerobe
- Catalase negative
- Beta hemolysis on human blood bilayer-tween agar
- Positive hippurate

Isolation of *Gardnerella* in culture is no longer considered appropriate to diagnose BV
“Whiff Test” (Amine Test)

Place vaginal fluid on slide
Add 1 drop of 10% KOH
Production of fishy odor is positive
Odor is caused by the production of amines
Amsel Criteria

Proposed by Richard Amsel et al., 1983

Uses the evaluation of 4 tests/observations

• Thin/homogeneous discharge
• Presence of clue cells on gram stain
• Vaginal fluid pH >4.5
• Positive ‘Whiff test’

Positive for 3 or 4 criteria is considered positive for BV

Positive for 2 to 4 criteria is considered positive for BV using modified criteria version
Ison/Hay Criteria

Uses Microscopy alone

• Grade 1 = Normal
  o *Lactobacillus* morphotypes predominant bacteria

• Grade 2 = Intermediate
  o Mixed flora with both *Lactobacillus* and *Gardnerella* or *Mobiluncus* morphotypes present

• Grade 3 = Positive for BV
  o *Gardnerella* or *Mobiluncus* morphotypes predominant and few or no *Lactobacillus* morphotypes
## Nugent Score Criteria

Obtain a score for each organism morphotype
Total all three scores for the “Nugent Score”

<table>
<thead>
<tr>
<th>Nugent Score Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Numbers of organisms per oil immersion field)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>&lt;1</th>
<th>1–4</th>
<th>5–30</th>
<th>&gt;30</th>
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<tbody>
<tr>
<td>Lactobacillus</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gardnerella &amp; anaerobic GNR</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Mobiluncus</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
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</table>
Nugent Score Criteria

<table>
<thead>
<tr>
<th>Nugent Score Interpretation</th>
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<tr>
<td>Nugent Score</td>
</tr>
<tr>
<td>1–3</td>
</tr>
<tr>
<td>4–6</td>
</tr>
<tr>
<td>7–10 (regardless of clue cells)</td>
</tr>
</tbody>
</table>

What should be done with intermediate findings?
Nugent Score Criteria (modification)

<table>
<thead>
<tr>
<th>Nugent Score Interpretation</th>
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</thead>
<tbody>
<tr>
<td><strong>Nugent Score</strong></td>
</tr>
<tr>
<td>1–3</td>
</tr>
<tr>
<td>4–6 (no clue cells present)</td>
</tr>
<tr>
<td>4–6 (clue cells present)</td>
</tr>
<tr>
<td>7–10 (regardless of clue cells)</td>
</tr>
</tbody>
</table>
OSOM BVBlue Test

Detects elevated sialidase activity in vaginal fluid
1 minute hands-on time; 10-minute read time
CLIA waived, no expertise required
Sensitivity 92.8%; specificity 98%
Reduced subjectivity
Limit of detection is 7.64 U
OSOM BVBlue Test

Sialidases are enzymes produced by some bacteria, fungi, mycoplasma, and protozoa.

Average level found in vaginal fluids of healthy controls = 2.7 U

Average level found in patients with BV = 12.3 U

Plays part in many cellular interactions:

- Defense to immune response
- Improved adhesiveness
- Destroys mucosal tissue
OSOM BVBlue Test

1. Collect a vaginal fluid sample with a swab. Contact the swab with the lower one-third of the vaginal wall. Collect as much fluid as possible. Put the swab into the BV Test Vessel. Gently swirl the mixture.

2. Let the BV Test Vessel containing the swab stand for 10 minutes between 17°C and 37°C.

3. Add one drop of Developer Solution to the BV Test Vessel containing the swab.

4. Gently swirl the mixture. Read the results immediately.

Positive Result: A blue or green color in the BV Test Vessel or on the head of the swab.


INTERPRETATION OF TEST RESULTS:
There are two possible results:
(a) positive result or (b) negative result.

NOTE: You may need to remove the swab to read the test results.

A Positive Result shows a high level of sialidase activity. A Negative Result shows a normal level of sialidase activity.
## Myziuk, 2003

Compared to Nugent score

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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</thead>
<tbody>
<tr>
<td>BVBlue</td>
<td>57</td>
<td>91.7</td>
<td>97.8</td>
<td>91.7</td>
<td>97.8</td>
</tr>
<tr>
<td>Amsel criteria</td>
<td>57</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>88.2</td>
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<tr>
<td>Abnormal vaginal discharge</td>
<td>31</td>
<td>58.3</td>
<td>46.7</td>
<td>22.6</td>
<td>80.8</td>
</tr>
<tr>
<td>Vaginal fluid pH &gt;4.5</td>
<td>12</td>
<td>66.7</td>
<td>91.1</td>
<td>66.7</td>
<td>91.1</td>
</tr>
<tr>
<td>Vaginal fluid amines</td>
<td>7</td>
<td>50</td>
<td>97.8</td>
<td>85.7</td>
<td>88</td>
</tr>
<tr>
<td>Clue cells</td>
<td>11</td>
<td>91.7</td>
<td>100</td>
<td>100</td>
<td>97.8</td>
</tr>
</tbody>
</table>
Compared BVBlue to Nugent score

288 subjects (all reported abnormal vaginal discharge or odor)

38% diagnosed with BV (7–10 Nugent score)

Sensitivity 88%

Specificity 95%

Bradshaw, 2005
Compared OSOM BVBlue to Amsel’s criteria and Nugent Score

347 subjects, 149 diagnosed with BV

Sensitivity 38.1% (differs substantially from most studies), specificity 92.7%

Reduced sensitivity observed in this study speculated to be result of different biota in the study population

Supports need for individual labs to validate tests prior to implementation

Madhivananan, 2014
BD Affirm VPIII Microbial Identification Test

Uses hybridization probes targeted to *Gardnerella vaginalis* nucleic acid

Also targets *Candida* and *Trichomonas*, providing three separate results

Limit of detection for *Gardnerella vaginalis* is $2 \times 10^5$ CFU
BD Affirm VPIII
Microbial Identification Test

Test Procedure for Candida, Gardnerella and Trichomonas
Always label Sample Collection Tube, Probe Analysis Card, and Reagent Cassette with correct sample ID.

Sample Preparation

1. Add 12 drops (0.4 mL) lysing solution and vigorously mix each sample for 10 seconds.
2. Incubate 10 min. at 37°C.
3. Add 12 drops (0.8 mL) buffer solution.
   - Tuck tube 10 times and then dispose of needle.

Automated Processing

1. Add 4 drops (0.1 mL) substrate solution to Well 7.
2. Place filter tip on tube.
   - Place tube in reservoir and dispense entire sample into Well 1.
3. Remove tube.
   - Place NAC in Well 1.
   - Start Processor
   - Remove NAC and interpret results.

Results

- **Positive**: Any blue color
- **Negative**: No blue color

Controls

- Test is **VALID** if:
  - Positive Control = Blue
  - Negative Control = Colorless

Qualitative results only. Positive results may be tighter or darker than the procedural control.
When Gram stains are performed to make the laboratory diagnosis of bacterial vaginosis, the smear is scored and interpreted according to published criteria.

NOTE: Culture should not be used for the diagnosis of bacterial vaginosis. Bacterial vaginosis (BV) is a syndrome involving a shift in the concentrations of aerobic and anaerobic flora of the genitourinary tract flora from a predominant presence of *Lactobacillus* sp. to that of a mixture of anaerobes, *Gardnerella vaginalis* and other gram-negative bacteria. Culturing for a particular organism, such as *Gardnerella vaginalis*, or any single organism or combination of organisms is not specific for the diagnosis of BV. Use of a scored Gram stain that demonstrates whether there has been a shift in the vaginal flora from predominantly gram-positive *Lactobacillus* to a gram-negative flora has been shown to correlate well with the Amsel criteria for the diagnosis of BV. The primary reason for performing a Gram stain on vaginal secretions is to diagnose bacterial vaginosis.

REFERENCES


Audience Poll

Of the tests your laboratory performed last month for bacterial vaginosis, approximately what percentage yielded a positive result?

- 0–10
- 11–25
- 26–50
- 51–75
- 76–100
Audience Poll

Of the tests your laboratory performed last month for bacterial vaginosis, approximately what percentage yielded a positive result?

N = 70
Bacterial Vaginosis Diagnosis: Research and Future Directions
Sha, 2005

Used quantitative real-time PCR to estimate organism loads

406 cervicovaginal lavage samples from repository

*Lactobacillus spp, Gardnerella sp, and Mycoplasma sp*

Concluded that quantitative PCR has potential as tool for evaluating vaginal flora

30% remained undiagnosed
Menard, 2008

Used quantitative real-time PCR to estimate organism loads

204 women from two locations in France; all subjects were pregnant

8 organisms (including *Lactobacillus sp*, *Atopobium sp*, *Mobiluncus sp*, *Gardnerella sp*, *Mycoplasma sp*, and *Ureaplasma sp*)

Using cutoff of $\geq 10^8$ *Atopobium sp* and $\geq 10^9$ *Gardnerella sp* offered best sensitivity, specificity, and predictive values
Wang, 2014

580 subjects, 230 BV positive by Nugent/Amsel

Quantitative PCR for 5 bacteria (Gardnerella, Atopobium, Leptotrichia, Megasphaera, and Mobiluncus)

High sensitivity (97%) and specificity (95%) when A. vaginae loads of 247,800 copies/ml used as cutoff value

Concluded A. vaginae load testing may be good diagnostic option in future
151 subjects tested by PCR for *Atopobium, Gardnerella, Megasphaera, and Lactabacillus*

Established ‘L-index’ to compare quantities of *Lactobacillus crispatus* (associated with healthy microbiota) and *Lactobacillus iners* (associated with altered microbiota)

Lower L-index and higher quantities of above organisms were found in subjects with NS of 7–10

Method has potential for automated, non-subjective diagnostic tool
Conclusions

BV can cause serious complications if untreated.

Nugent score gold standard is subjective, not available POC, and yields a high number of ‘intermediate’ results.

BV appears to be associated with several different bacterial species, not just *Gardnerella vaginalis*, making test development difficult.

Using condition markers such as sialidase, clue cells, or multiple target bacterial loads offers testing alternatives to Nugent score gram stain.

More research is needed.
Audience Poll

What method does your lab use to test for bacterial vaginosis?

- Affirm VPIII test
- Gram stain reported with Nugent score
- OSOM BVBlue test
- Other
Audience Poll

What method does your lab use to test for bacterial vaginosis?

- Affirm VPIII test: 24.3%
- Gram stain reported with Nugent score: 10.8%
- OSOM BVBlue test: 4.1%
- Other: 60.8%

N = 74
Q&A

To type in a question, navigate to the Q&A section of the event panel.
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www.sekisuidiagnostics.com
Contacts

Donna Mayne, BS, (MT)ASCP, (CLS)NCA
dmayne@shhpens.org
850/416-7772

Steve Halasey
shalasey@allied360.com
626/219-0199
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