Bacterial Vaginosis: Pathogenesis, Presentation, and Diagnosis

Don Stalons, PhD, D(ABMM), MPH
Dir., Clinical Laboratory, Diatherix
Learning Objectives

- Review the role of both pathogenic and commensal microbiota involved in urogenital infections
- Identify the methods to detect a broad range of vaginal microbiota from a single sample quickly, and cost effectively
- Discuss the practical application of the detection of a broad range of vaginal microbiota from a single sample
The Microflora of Mucosal Surfaces of the Human Body in Health and Disease

Protection

- Normal flora
  - Layer formation
  - Waste product formation
  - Immune stimulation

Pathologic flora

Disease

- Large inoculum
- Antimicrobial drugs
- Host factors
- Physical destruction
The Normal Microflora of the Vaginal Tract

- Predominant flora of lactobacilli species that colonize and secrete chemical products.
- An ecosystem that harbors a microbiota that protects it from invading pathogens including those that cause urinary tract infections and sexually transmitted diseases.
- Lactobacilli are dominant at concentrations of $10^7$ to $10^8$ CFU/g of vaginal fluid.
- Potential pathogens are kept at insignificant levels due to the production of large volumes of lactic acid and hydrogen peroxide.

Prominent factors that may predispose patients to BV include:

- Recent antibiotic usage
- Decreased estrogen production of the patient
- Wearing an intrauterine device (IUD)
- Douching
- Sexual activity that could lead to transmission (e.g. having a new sexual partner or a recent increase in the number of sexual partners)

Investigating Bacterial Vaginosis

BV is one of the most widely studied obstetric/gynecologic infectious diseases and may affect 1/3 of women at some point in their lives

- Vaginal odor (the most common, and often initial, symptom of BV)
- Mild to moderate increase in vaginal discharge
- Vulvar irritation (not always present)
- Dysuria or dyspareunia are rare
- Disease is more prevalent in certain races and lower socioeconomic classes

These signs and symptoms were summarized by Amsel in 1983 and have become the clinical standard for BV diagnosis

- Vaginal pH > 4.5
- Presence of > 20% per HPF of “Clue cells” on wet mount examination
- Positive amine or “whiff test”
- Homogeneous, non-viscous, milky-white discharge adherent to the vaginal wall
Standardized Microscopic Technique for the Determination of Bacterial Vaginosis

Laboratory examination of vaginal smears and the determination of the Nugent Score

N Score = Sum of the scores for each bacterial morphotypes listed below (Note number of Organisms seen/100 X objective)

<table>
<thead>
<tr>
<th>Lactobacilli</th>
<th>Score</th>
<th><em>Gardnerella, Bacteroides</em></th>
<th>Score</th>
<th>Curved Gram-negative bacilli</th>
<th>Score</th>
<th>Sum = Nugent Score</th>
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**Interpretation of Nugent Score**

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<td>Smear NOT consistent with BV</td>
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<tr>
<td>4-6</td>
<td>Clue Cells ARE Present</td>
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<tr>
<td>4-6</td>
<td>Clue Cells ARE Present</td>
<td>Smear consistent with BV</td>
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Aerobic Vaginitis

Distinguishing Features and Determination
Aerobic Vaginitis Differs from BV

- Discharge more colored and viscous
- Depletion of normal concentrations of Lactobacilli (very low numbers in more severe cases)
- Discharge is foul smelling (not the typical amine odor with BV)
- Histological appearance of desquamative inflammatory vaginitis (most severe form)
- Significant immune cytokine response (IL-1-β and IL-6)
- Cultures of often purulent discharge show Staph spp, Group B Strep, enterococci, and Gram neg bacilli (often E. coli and Klebsiella)

### Presentation and Complications

- Patients complain of burning or painful sensation during intercourse
- Vulvar or vaginal itching
- Ulcerations often associated with moderate to severe case
- Aerobic vaginosis more likely associated with pregnancy complications
- Ascending chorioamnionitis
- Preterm rupture of the membranes
- Preterm delivery

### Determination

- Yellow swab test
- Increased pH (> 6)
- Microscopy of wet mount
- Foul smell of discharge without application of KOH
- More esoteric tests include evaluation of cytokines (not feasible for routine practice)
- Other than those mentioned above, point of care testing is limited
The Need for Better Determination in Bacterial Vaginosis and ‘Aerobic’ Vaginitis

- Both disease entities often rely on subjective criteria of microscopic analysis
- Both diseases are associated with an increased risk of other sexually transmitted diseases
- These clinical entities cannot be treated and managed in the same way (see below)
- Both diseases can affect the outcome of pregnancy; often with severe consequences

### Implicated Sexually Transmitted Diseases
- Chlamydia
- Gonorrhoeae
- Trichomonas
- Mycoplasma
- Ureaplasma
- HIV
- Herpes simplex I and II

### Treatment Regimens
- **Bacterial Vaginosis** = Metronidazole or Clindamycin and restoration of Lactobacilli
- **Aerobic Vaginosis** = Kanamycin ovule, 2% Clindamycin topical, Ampicillin, Fluoroquinolones
Application and Unique Advantages of Molecular Technology

Identification of key organisms associated with vaginosis/vaginitis

Lactobacilli
Commensal organisms associated with colonization of the vaginal mucosal surface and are effective producers of $H_2O_2$ to suppress the growth of invading pathogens include:

- *L. crispatus*
- *L. gasseri*
- *L. jensenii*
- *L. iners*
- *Clostridiales* order (BVAB 1-3)
- *Atopobium vaginae*
- *Gardnella vaginalis*
- *Sneathia/Leptotrichia*
- *Megasphaera* types 1 and 2
- *TM7* type bacteria

Organisms that are associated with both BV and Aerobic Vaginitis

- *Clostridiales* order (BVAB 1-3)
- *Atopobium vaginae*
- *Gardnella vaginalis*
- *Sneathia/Leptotrichia*
- *Megasphaera* types 1 and 2
- *TM7* type bacteria
- Group B Strep
- Staph sp
- *E. coli*
- *Enterococcus* sp

- Traditional methods are subjective and lack sensitivity/specificity\(^1\)
- Reproducible molecular techniques are more empirical
- Panel-based or multiplex formats permit simultaneous detection of multiple targets associated with various conditions

\(^1\)Bacterial Vaginosis: An Update on Diagnosis and Treatment; Expert Rev Anti Infect Ther. 2009 Nov;7(9):1109-24
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Vaginal Microbiota Testing using real-time PCR

Doug Rains, Chief Scientific Officer
Quantigen
Introduction

• **Main goals:**
  - To accurately ID pathogenic organism as quickly as possible.
  - To identify **co-infections** possibly requiring multiple drugs.
  - Ideally: identify any antibiotic resistance (future goal).
Finding a Better Approach

- **Ideal wish list** for future infectious disease test:
  - High level of **specificity** (preferably species-level information) and **sensitivity**.
  - **Broad coverage** of the various microorganisms that can cause a similar presentation.
  - A **single**, minimally invasive **sample collection**.
  - **Rapid** turnaround times.
  - **Low cost** per sample and **easy workflow**.
Real-time PCR Solution

➢ Panel Testing Using Real-Time PCR
  ▪ Many benefits:
    • **Specificity** – Real-time PCR reports precisely which microorganism(s) are present.
    • **Sensitivity** – Numerous CDC-acknowledged studies have demonstrated the improved detection rates of PCR-based methodologies over more traditional tests, especially for STIs.*

*Source: Morbidity and Mortality Weekly Report, Centers for Disease Control; March 14, 2014 / 63(RR02); 1-19.
Panel Testing Using Real-Time PCR

- Many benefits:
  - **Broad coverage** – Real-time PCR comprehensive test covers 27 microorganisms from four major areas:
    - Sexually-transmitted infections (STIs)
    - Aerobic vaginitis (AV), including Group B Strep
    - Candidiasis
    - **Bacterial vaginosis** (details to follow)
Panel Testing Using Real-Time PCR

Many benefits:

- **Flexibility** – One can choose which individual panel(s) to run, or perform a comprehensive screen in cases where either (a) presentation profile is vague or ambiguous, or (b) multiple infections are suspected.
Panel Testing Using Real-Time PCR

- Many benefits:
  - **Single sample collection** – Panel-based real-time PCR performs a fully comprehensive screen across all 27 organisms using a single vaginal swab.
Real-time PCR Solution

Panel Testing Using Real-Time PCR

- Many benefits:
  - **Rapid turnaround**– The ability to report full-panel results within 12-24 hours of sample receipt.
Utilizing Real-Time PCR System:

- Uses 5’ nuclease chemistry (sensitive and specific).
- All assays on this panel were pre-designed.
- Low cost per data point / sample
- Easy workflow and quick TAT.
- Max. throughput: about 180 samples/8-hour shift.
Developing a real-time PCR bacterial vaginosis test

- **Studying Bacterial vaginosis: background**
  - A condition in which the vaginal microflora, normally dominated by lactobacillus spp., is overtaken by an array of **anaerobic species**.
  - Often **asymptomatic**; when symptoms are present, most are non-specific (e.g., itching, discharge).
Developing a real-time PCR bacterial vaginosis test

- **Big challenges:**
  - No single microbe is exclusively associated with BV.
  - Heavy reliance on presentation: *Amsel criteria* most common approach.
    - Questionable sensitivity, no accounting for *lactobacilli*.
  - Alternatively, experienced labs can prepare a Gram-stained sample: *Nugent testing*.
    - Requires experience, can be time-sensitive, and is blind to species with no cell wall (e.g., mycoplasma).
Developing a real-time PCR bacterial vaginosis test

Better approach:

- Characterize vaginal microflora using a highly **specific and sensitive method**, such as real-time PCR / 5’ nuclease chemistry.
- Survey a **large collection of microbes** known to associate with BV.
- Determine which **microbial signatures** associate with BV (using Nugent scoring as the benchmark).
- Develop an **interpretive algorithm** that is as independent of sample collection technique as possible.
Microbes assessed

- *Gardnerella vaginalis*
- *Atopobium vaginae*
- Megasphera Type 1
- Megasphera Type 2
- *Mycoplasma hominis*
- *Mobiluncus curtisii*
- *Mobiluncus mulieris*
- *Ureaplasma urealyticum*
- BVAB2
- *Prevotella bivia*
- *Lactobacillus iners*
- *Lactobacillus jensenii*
- *Lactobacillus gasseri*
- *Lactobacillus curtisii*
- Broad-range 16s (for monitoring sample collection)
**Real-time PCR BV Study Design**

- **Phase 1a: algorithm training**
  - Collect 200 duplicate study swab samples – one for Nugent, one for real-time PCR using large BV panel.
  - Data → Bioinformatics partner for statistical analysis / development of a **PCR-based interpretive algorithm** that predicts BV status.
Real-time PCR BV Study Design

- **Phase 1b: algorithm validation**
  - Collect an additional 200 study samples for Nugent / real-time PCR.
  - Apply algorithm developed in Phase 1a.
  - Calculate **specificity and sensitivity** relative to Nugent scores.
# Example of Results

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<th>Megasph. 2</th>
<th>M. hominis</th>
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<th>M. curtisi</th>
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<th>M. mulieris</th>
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Sensitivity
= percentage of samples correctly identified as BV+

Specificity
= percentage of samples correctly identified as BV-
Sorted by Nugent Score

CLS: 50
Nugent: 59

84.7%

Sorted by Nugent Score

CLS: 90
Nugent: 95

94.7%

Sensitivity
= percentage of samples correctly identified as BV+

Specificity
= percentage of samples correctly identified as BV-
Sorted by Nugent Score

Sensitivity

= percentage of samples correctly identified as BV+

Specificity

= percentage of samples correctly identified as BV-
Real-time PCR BV Study: Phase 2

Phase 2 (ongoing)

- Collect an additional 200 study samples for Nugent / real-time PCR, including 50 negative subjects.
- In addition to Nugent scoring, attain putative BV status using both Amsel criteria and a secondary molecular method.
- Refine algorithm in an effort to achieve greater specificity and sensitivity.
Real-time PCR BV Study: Collaborators

A collaboration between Quantigen Laboratory in Fishers, IN, PrimeX Laboratories in Van Nuys, CA, and the Research and Development Institute (RDI) in Van Nuys, CA.

Acknowledgments:

Erik Avaniss-Aghajani, PrimeX Laboratories
Zohrab Bostanian, RDI
Coriell Institute for Medical Research
Statement

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Developing a Molecular Assay Algorithm for Bacterial Vaginosis Determination

Jeffrey Shaman, PhD, Dir., Business Development, Coriell Life Sciences
Women’s Health Reporting

<table>
<thead>
<tr>
<th>Data</th>
<th>Report Requirements</th>
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<td>1. Results from Real-time PCR Women’s Health microorganism assay</td>
<td>• Results of assays</td>
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<td>Infection Information</td>
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<td>2. Infection details and demographics from the Study</td>
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Coriell Life Sciences

- Borne from the 64 year old Coriell Institute for Medical Research
- Focused on empowering those who use genetics
- Support laboratories in their missions to deliver world-class molecular solutions
- Leader in Laboratory Reporting, Decision Support Tools, Medical Therapy Management, Medical Risk Reporting, Genetic Interpretation, and Pharmacogenomics
Women’s Health Reporting

**Goal**
- Identify an Algorithm that can Recognize Patterns in the WH microorganism assay results from Real-time PCR

**Success Metric**
- The algorithm’s output matches the Study’s assessment of positive Bacterial Vaginosis

**Report Requirements**
- Results of assays
- Microorganism Details
- Interpretation
- Determination of BV
Women’s Health Reporting

Report Requirements

• Results of assays
• Microorganism Details
• Interpretation
• Determination of BV
Women’s Health Reporting
Algorithm Development Process

• Ingest raw data
  – 400 samples
• Convert raw data into usable form
  – Linearize Ct
• Apply statistical models to a training subset
  – (linear, logarithmic, orthogonal transformations, etc.)
• Evaluate wide variety of models—apply algorithm to validation set of data
• Choose model that meets success metrics
**Women’s Health Reporting**

Algorithm Development Process Raw Data

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Women’s Health Reporting
Algorithm Development Process Raw Data
Evaluated 53 Distinct Models clustered the samples

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All 14 Bacteria
Sensitivity: 74.4%
Specificity: 95.1%

Bacteria, Age, History of Vaginitis;
Positive whiff test; Pregnant.
Sensitivity: 75.6%
Specificity: 98.6%

Orthogonal Transformation
### Women’s Health Reporting

Algorithm Development Process Raw Data

Convert Data for Evaluation of Orthogonal Transformation Model

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Convert set of observations into a set of values
The CLS1 High group contains only 5% of Nugent Lows; those 5 samples “look” pathogenic

- CLS1 High = BV+

The CLS1 Lows are Nugent Lows with a mix of additional Nugent categories (Intermediates, Highs)

- CLS1 Low = Not conclusive for Bacterial Vaginosis

“Exceptions” to the CLS1 Algorithm make biological sense
Investigation outcomes: The pattern of pathogenic and normal bacterial flora does not suggest bacterial vaginosis.
CLS1 Algorithm Result = HIGH

• Interpretation: The pattern of pathogenic and normal bacterial flora suggests bacterial vaginosis.
Women’s Health Reporting
Simple Reporting Process

Lab Receives CLIA Report
Lab posts data
Process and Architecture

Reports

Infrastructure

Customers

Genetic data

Laboratories, Hospitals, ACO’s etc

GeneVault

Ingestion Engine

GeneExchange

PGx Interpretation Algorithm

Reporting Engine

GeneDose

GDL SafeTRx

Reports

Customers
Process and Architecture

Reports

Infrastructure

Customers

GeneVault

Ingestion Engine

GeneExchange

PGx Interpretation Algorithm

WH Interpretation Algorithm

Reporting Engine

Antibiotic Resist Panel

Colon Cancer Diag.

Cystic Fibrosis Diag.

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Laboratories, Hospitals, ACO’s etc

Laboratories, OBGYN Clinics

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Laboratories, Hospitals, ACO's etc

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 PGx Interpretation Algorithm
 WH Interpretation Algorithm
 CF Interpretation Algorithm
 Antibiotic Resistance Algorithm
 Colon Cancer Algorithm

GeneVault

GeneExchange

Ingestion Engine

Ingestion Engine

GDL
 SafeTRx

Reports

Infrastructure

Customers
Women’s Health Reporting

• Support laboratories in their missions to deliver world-class diagnostic solutions
• Complete Decision Support
• Actionable based on Study with Quantigen and ThermoFisher Scientific
• Customizable reports – logos, branding, and panels