Overview of Wet Preps and Gram stains

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A secondary objective of the 035 study is to assess the effectiveness of BufferGel and Pro2000 in preventing bacterial vaginosis.

BV has been shown to be a risk factor for acquiring HIV, gonorrhea, and HSV-2.

BV is a common cause of vaginal symptoms putting a large number of women at a higher risk for acquiring HIV than women with normal flora.
Women with normal flora benefit from the bi-products of *Lactobacillus*.

- Lactic acid helps maintain a low vaginal pH.
- Hydrogen peroxide *in vitro* inhibits HIV.

Women colonized with H$_2$O$_2$ producing *Lactobacillus* are less likely to have a shift in flora.
Methods for Diagnosis of Bacterial Vaginosis

1. Clinical Diagnosis, Amsel criteria
   - This will be used to clinically manage patients

2. Gram stain, Nugent score
   - Centralized and standardized lab based method
   - Standard for clinical trails
   - Not used for clinical management
Clinical Diagnosis

Must have 3 of the 4 signs for a diagnosis of BV
- Discharge, white & homogeneous
- Elevated pH, >4.5
- Amine odor present
- 20% clue cells present on wet mount
Specimen Collection

- Swab the lateral or posterior fornices of the vagina
- Place the swab in a sterile tube containing ~6 drops of saline
- To assure the consistency of the volume of saline the lab should provide the clinic with the saline tubes.
- Transfer to the lab ASAP.
“Decreasing Shelf-life” of *Trichomonas vaginalis* on Wet Mount Preparations

20% of wet mounts initially positive for *T. vaginalis* become negative within 10 minutes

Ref: Kingston MA, Bansal D, Carlin EM. Int J STD and AIDS 2003; 14: 28-29
Evaluation of PCR, Culture, Wet Mount, and Pap Test for the Diagnosis of Trichomoniasis

1. Discharge

- White & homogeneous
- Limitations: douching or cleansing prior to exam, small amount of discharge
- Use of microbicide or placebo gels may make evaluation of discharge more difficult.
Clinical Diagnosis

2. pH of vaginal fluid
   - Use S/P pH Indicator Strips Range 3.6 to 6.1
   - Touch the strip to the wall of the vagina or use a swab of the vagina to moisten the pH paper.
   - Avoid the cervical mucus, which has a higher pH than the vagina.
   - Compare the color to the chart on the container.
   - pH >4.5 is consistent with BV
   - Limitations: blood, sperm, and cervical mucus can elevate the pH; acid gels may lower the pH.
3. Amine odor

- KOH prep
- Transfer a small amount of the vaginal fluid onto a slide.
- Add 1 drop of 10% KOH to one of the slides.
- A fishy odor indicates the presence of amines.
- Limitation: technologist may not be able to smell the odor.
- Cover slip the slide and read for budding yeast and pseudohyphe.
Clinical Diagnosis

4. Wet Mount

– Adhere a SCHARP label on the slide.
– Transfer a small amount of the vaginal fluid onto a slide.
– Add a drop of saline if there isn’t enough fluid and mix.
– Cover slip the slide.
Clinical Diagnosis

- Initially, read at 10X for *Trichomonas* and yeast.
- Scan the whole coverslip for *Trichomonas*.
- Read the epithelial cells at 40X to determine if clue cells.
- For the 035 study there must be 20% clue cells to be positive for clue cells.
- WBC are not recorded for the 035 study, but may be requested by the clinician.

**Limitations:**
- Presence of gel may interfere with observation.
- Important to read for *Trichomonas* within 1 hour.
Quality Control

- **pH paper**
  - If there isn’t an outdate on the pH paper, write a date of 6 months on the container.
  - Check with certified buffers pH 4 and pH 6 once a month.

- **Wet mount**
  - Duplicate reading once a week to show reproducibility

- **KOH**
  - Check monthly with pH paper to assure acidity.
Reports

- The source document is the first place the results are recorded.
- Include the date and time of receipt and testing. The time is critical for the wet mount.
- Signature of testing technologist.
- Signature of reviewer.
Proficiency Testing of Wet Mount Reading

- Provides an outside assessment of the accuracy of the laboratory’s ability to perform a specific test.
- CAP does not provide a panel of tests exclusively for vaginal wet mounts.
- The central lab will provide a panel of 5 photographs twice a year via e-mail.
- The results from each lab will be sent to the central lab.
Gram Stain for Diagnosing BV

- Based on the quantity of 3 morphotypes
  - Lactobacilli, Gram variable rods, and curved rods
- Sensitivity 86%-89% and specificity 94%-96% compared to Amsel criteria
- Will be done at Central Lab in Pittsburgh
Specimen Collection

- Label 2 clean slides with the Scharp label.
- Swab of the vaginal fluid is rolled onto the slide.
- Air dry and store in slide boxes.
- Enter into LDMS but **do not use** LDMS labels on the slides.
- Twice a year one set of slides will be sent to the central lab at the Pittsburgh location.
- Also send the LDMS manifest file for each box of slides.
Scoring of Gram Stain

- Slides are scanned on 10X to evaluate the quality of the smear. (Must have epithelial cells)
- Bacterial morphotypes are quantitated.
Quantitation of Bacterial Morphotypes

- 0-1/1000X magnification = 1+
- 1-4/1000X magnification = 2+
- 5-30/1000X magnification = 3+
- >30/1000X magnification = 4+

Read at least 5 nonconsecutive fields and average the quantity of morphotypes.
# Nugent Scores of Gram Stain

<table>
<thead>
<tr>
<th>Morphotype</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+ Lactobacilli</td>
<td>0</td>
</tr>
<tr>
<td>3+ Lactobacilli</td>
<td>1</td>
</tr>
<tr>
<td>2+ Lactobacilli</td>
<td>2</td>
</tr>
<tr>
<td>1+ Lactobacilli</td>
<td>3</td>
</tr>
<tr>
<td>0 Lactobacilli</td>
<td>4</td>
</tr>
<tr>
<td>1+ Gardnerella/Ana GNR</td>
<td>1</td>
</tr>
<tr>
<td>2+ Gardnerella/Ana GNR</td>
<td>2</td>
</tr>
<tr>
<td>3+ Gardnerella/Ana GNR</td>
<td>3</td>
</tr>
<tr>
<td>4+ Gardnerella/Ana GNR</td>
<td>4</td>
</tr>
<tr>
<td>1-2+ Mobiluncus</td>
<td>1</td>
</tr>
<tr>
<td>3-4+ Mobiluncus</td>
<td>2</td>
</tr>
</tbody>
</table>
Points are added according to the morphotypes seen.

Total will be between 0 and 10

- **0-3** Normal
- **4-6** Intermediate
- **7-10** bacterial vaginosis
Quality Control

- The label on the slide is compared to the manifest file.
- 10% of the slides are read by a second technologist.
- The final report is compared to the source document prior to sending to Sharps.
Reporting

- Manifest file sent from the sites is used as a template for the results.
- Quantity of WBCs and the Nugent score are entered into the manifest file and sent to Sharps.
- A hard copy of the source document and manifest report are kept on file.
- The manifest reports are backed up on the network server and on CD.
Inventory of Slides

Date received
Site name
Study number
Number of slides
Date report sent to Sharps