Gram Stain Review

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Objectives

- To discuss:
  - Laboratory diagnosis of *Neisseria gonorrhoeae*
  - Specimen collection
  - Gram Stain Test Procedure
  - Diagnosis of Gonorrhea
  - Gram Stains
  - Microscopy Review

*Neisseria gonorrhoeae*

- *N. gonorrhoeae* is the causative agent of gonorrheal disease.
- Non-motile, gram-negative diplococci
- The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left untreated in woman, it can cause PID which can lead to infertility.
Urethral specimens:

- The patient should not have urinated for at least one hour prior to specimen collection. Use only the specimen collection swab provided (blue shaft swab) to collect specimen. Insert the blue swab 2-4 cm into the urethra. Rotate for 2-3 seconds. Withdraw swab and place into the specimen transport tube. Break the swab at the scoreline. Recap the tube tightly. The swabs are suitable for smear preparation, culturing on appropriate media or for transport to other laboratories.

Urine Specimens:

- Urine is one of the specimen types suitable for nucleic acid tests for diagnosing N. gonorrhoeae and Chlamydia trachomatis infections in males and females. Leak-proof containers should be provided to patients for the collection of urine specimens and collected according to lab requirements.
- The patient should not have urinated at least one hour prior to specimen collection.
- Direct patient to provide only the first-catch urine of 20 to 30 ml of initial urine stream, do not cleanse area, and collection of larger volumes may result in specimen dilution that could reduce test sensitivity.

Oropharyngeal and Rectal Specimens:

- Oropharyngeal and rectal specimens should be processed only for culture on a Jembec plate because the performance of nonculture methods is not well established for these specimen types.
- Inoculated plates should be incubated at 35°C to 37°C in a moist atmosphere enriched with a CO₂ tablet for at least 18-24 hours.
Diagnosis of Gonorrhea

- A Presumptive diagnosis of gonorrhea is made on the basis of one of the following three criteria:
  - typical gram-negative intracellular diplococci on microscopic examination of a smear of urethral exudate from men
  - growth of a gram-negative, oxidase-positive diplococcus, from the urethra (men) or endocervix (women), on a selective culture medium, and demonstration of typical colonial morphology, positive oxidase reaction, and typical gram-negative morphology.

Diagnosis of Gonorrhea

- detection of N. gonorrhoeae by a nonculture laboratory test (Antigen detection test (e.g., Gonozyme [Abbott]), direct specimen nucleic acid probe test (e.g., GenProbe), nucleic acid amplification test.

Gram Stain Test Procedure:

1. Flood the fixed smear with primary stain (Gram Crystal Violet) and stain for 1 min.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant Gram Iodine and retain on the slide for 1 min.
4. Remove the mordant by gently washing with tap water.
5. Decolorize (Gram Decolorizer) until solvent running from the slide is colorless (3 – 60 sec).
6. Wash the slide gently in cold tap water.
Gram Stain Test Procedure:

7. Flood the slide with counterstain (either Gram Safranin or Gram Basic Fuchsin) and stain for 30 – 60 sec.
8. Wash the slide with cold tap water.
9. Blot with blotting paper or paper towel or allow to air dry.
10. Examine the smear under an oil immersion lens.

• User Quality Control
• Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations.
A vaginal wet prep is a gynecologic test wherein a sample of vaginal discharge is observed by wet mount microscopy by placing the specimen in a saline solution and then placing a drop on a glass slide. It is used to find the cause of vaginitis and vulvitis.

The slide is looked at under a microscope for bacteria, yeast cells, trichomonas, white blood cells that show an infection, or clue cells that show bacterial vaginosis.

Evaluate the structure at the tip of the pointer in the Vaginal Wet Prep:
This structure looks different:

Vaginal Wet Prep:

Identify the Cells:
# Differential Diagnosis of Vaginal Infections

## Clue Cells:

![Image of Clue Cells](image1)

## Trichomonas Vaginalis:

![Image of Trichomonas Vaginalis](image2)

## Differential Diagnosis of Vaginal Infections

<table>
<thead>
<tr>
<th>Diagnostic Criteria</th>
<th>Normal</th>
<th>Bacterial Vaginosis</th>
<th>Trichomonas</th>
<th>Vulvo-vaginal Candidiasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal pH</td>
<td>3.8-4.2</td>
<td>Greater than 4.5</td>
<td>Greater than 4.5</td>
<td>Usually less than 4.5</td>
</tr>
<tr>
<td>Discharge</td>
<td>Thin, flocculent clear or white</td>
<td>Thin, homogenous white (milky) gray</td>
<td>Frothy or foamy White, yellow or green</td>
<td>Thick, clumpy, white “cottage cheese like”</td>
</tr>
<tr>
<td>Amine Odor (Whiff test)</td>
<td>Absent</td>
<td>Present (Fusty)</td>
<td>Usually Present (Fusty)</td>
<td>Absent</td>
</tr>
<tr>
<td>Microscopic Findings</td>
<td>Lactobacilli, Epithelial cells</td>
<td>Clue cells with Adherent Bacteria, No or few WBCs</td>
<td>Most Trich, WBCs greater than 10/hpf</td>
<td>Budding yeast, Hyphae, Pseudohyphae</td>
</tr>
</tbody>
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Acknowledgments

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Questions?

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