

<b>GRAM STAIN INTERPRETATION</b>
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**PURPOSE**

To provide instruction for the reading and interpretation of a gram stain.

**BACKGROUND**

The gram stain of bacteria is one of the most important determinations in the presumptive identification of microorganisms. The morphology of the bacterial cells, their arrangement and their staining characteristics are often distinct enough to allow a presumptive identification in a gram stained smear.

**RELATED DOCUMENTS**

- R-W-MB-707 Gram staining and preparation
- R-W-MB-404 Sputum adequacy

**STEPS**

1. All personnel must be trained to read gram stain smears. If rotation into Microbiology has not been on a regular basis or if there is any question about reading a smear, a QC slide must also be performed concurrently with gram stain testing. All techs reading gram stains must pass the yearly competency testing.
2. For all slides that appear inadequately prepared and the slide cannot be remade, scant specimen or contaminated enter Insufficient material for gram stain interpretation under the gram stain tab description.
3. Review entire slide, looking at multiple fields, before entering patient results.
4. Gram stain reactions:
  - Gram positive organisms stain deep violet to purple. Yeast are egg-shaped and usually larger than bacteria.
  - Gram negative organisms stain pink or pink-red.
  - Gram variable organisms stain partially gram positive and partially gram negative (an example would be Gardnerella sp)
  - Some organisms do not pick-up the stain and have a clear appearance. This can be seen with some fungi or Mycobacterium.
  - Staining characteristics can be even, bipolar, beaded, stippled or irregular.
  - Organism shapes are rods, cocci, coccal bacilli or diplococci and can be gram positive or gram negative. Some organisms occur in pairs, some occur in chains or clusters.
  - Be aware as the slide is being reviewed, that the stain may be under or over decolorized. Some organisms may not be their true color. In this case, review the shape of the organisms. Restaining the smear may be necessary.

5. Quantification of observed cells and organisms:
  - Rare = < 1 per oil immersion field
  - Few = 1 to 5 per oil immersion field
  - Moderate = 5 to 10 per oil immersion field
  - Numerous = > 10 per oil immersion field
6. The microbiology tech must evaluate the slides for adequacy and quality of specimen. The tech at the bench must use the gram stain slide interpretations to guide their workup of cultures. If an organism is present on the primary slide but not the culture, either review the smear or look for the organism and use selective media to grow the missing organism. There also may be an anaerobic organism or fastidious organism requiring special media to grow. If an organism does not grow that is on the smear, add a comment to the culture report the organism (s) found on the gram stain could not be isolated on culture.
7. For all gram stain slides read at other FHS labs, add in the Mnemonic column the location of where the slide reading was performed at. This information will be added to the patient's results and post in Epic and Beaker. If the slide is read at SJMC lab, the computer will automatically add SJMC to the results.

## RESULTS

### 1. Vaginal/Cervical smears

- A scoring system for the interpretation of genital smears may be used. The scoring system provides a 0-10 point scale for the evaluation of vaginal flora.
- Each organism morphotype is quantitated from 0-4+ with regards to the number of organisms present per oil immersion field.

0 = no organisms/oil immersion field  
 1+ = less than 1 organisms/oil immersion field  
 2+ = 1 to 4 organisms/oil immersion field  
 3+ = 4 to 30 organisms/oil immersion field  
 4+ = 30 or more organisms/oil immersion field

- Review the smear for large gram positive rods (Lactobacillus), grade 0-4+
- Review the smear for small gram negative or gram variable rods (Gardnerella/Bacteroides) grade 0-4+
- Review the smear for curved gram negative rods (Mobiluncus) grade 0-2+
- Determine the score for each organism morphotype and then add the three scores together.
- The interpretation of the smear is based on the sum of the scores:

0 to 3 = normal vaginal flora  
 4 to 6 = intermediate vaginal flora  
 7 to 10 = bacterial vaginosis  
 If only gpr (Lactobacillus) is present, this organism represents normal vaginal flora.

For example:

large gpr = 1+ (<1/oil field) score = 1  
 small gvr = 4+ (>30/oil field) score = 4

curved gnr = 3+ (4 -30/oil field) score = 2  
Total is 7, interpretation = bacterial vaginosis

## Entering Results

1. In the LIS, enter the patient's accession number under Result Entry. Click on Edit. Make sure you use the Stain tab.
  - Using oil, review the smear for WBC's and enter the appropriate code. Enter the quantity under the quantity column and use the description column for the WBC's. Use the magnifying glass to search for codes. If there are no WBC's, go to the description column and enter NO, search with the magnifying glass and all the answers starting with No will appear.
  - Look for epithelial cells if present. Enter the quantity under the quantity column and use the description column to enter Epithelial cells.
  - If Lactobacillus( gram positive rods) are present, enter the quantity and GPRL in the description.
  - If small gram neg/gram variable rods, resembling Gardnerella enter the quantity and GARD in the description column.
  - If curved gram negative rods resembling Mobiluncus are present enter the quantity and curved gram negative bacilli
  - If no Gardnerella or Mobiluncus are present, enter No Gardnerella present and No Mobiluncus present, search using No
  - Quantify and enter any other bacteria present also look for the presence of Neisseria gonorrhoeae (gram neg diplococci , kidney bean shaped pairs)
  - Quantify and enter the absence or presence of yeast. If yeast are present also look for hyphae. Enter No yeast seen, yeast seen, yeast with hyphae or yeast with pseudohyphae seen.
  - Clue cells are squamous epithelial cells heavily colonized with short, gram variable pleomorphic rods to such an extent that margins of the cells are indistinguishable. Clue cells are an indication of Gardnerella vaginalis infection. Enter Clue cells present or No clue cells present.
  - Enter the direct smear interpretation :
    - Normal vaginal flora (Lactobacillus, no Gardnerella, Mobiluncus, yeast or clue cells seen).
    - Shift in flora consistent with Bacterial vaginosis ( Gardnerella or Mobiluncus seen and clue cells present).
    - Shift in flora indeterminate for Bacterial vaginosis (Gardnerella or Mobiluncus or clue cells both not all)
    - If yeast are present, enter No direct smear interpretation due to yeast present.
    - If no organisms are present, enter No direct smear, vaginal flora absent.
  - For male specimens, enter cells and organisms seen. Do not enter direct smear interpretation.
  - Before Final verifying results, go to the Culture tab. Click on the magnifying glass and enter either Normal or Abnormal results according to results entered then final.

## 2. Smears for Neisseria gonorrhoeae

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- Smears from males and females are evaluated for gram negative diplococci, intracellular or extracellular. Report Few, Moderate or Numerous under the quantity column. Under the description column, enter gram negative diplococcic, intra or extracellular.

### 3. Smears for Respiratory Specimens

- Review the smear for adequacy (see R- W-MB 404, Sputum Adequacy).
- Using the low 10x objective, scan the entire slide. Review the slide for WBC's and enter No WBC's or quantitate Few, Moderate or Numerous and WBC's in the description column.
- Exam the slide for epithelial cells and enter the code for No epithelial cells or quantitate Few, Moderate or Numerous and Epithelial cells in the description column.
- Determine if the smear shows normal respiratory flora which may include:
  - gram positive cocci in pairs or chains
  - gram negative or gram positive rods
  - gram negative cocci
  - yeast
  - If no predominant organism is present, quantitate the amount present and enter the code mixed flora – no predominate organism.
- Haemophilus are small gram negative coccalbacilli. Quantitate and enter the code, gram negative coccalbacilli .
- Moraxella catarrhalis is a gram negative diplococci. Quantitate and enter the code gram negative cocci – Impression : increased Neisseria or Moraxella
- Streptococcus pneumoniae are lancet-shaped gram positive diplococci with encapsulation. A clearing can be seen around the pair of gram positive cocci. Quantitate and enter the code gram positive diplococci with encapsulation Impression: Strep. Pneumoniae
- Staphylococcus aureus are gram positive cocci in pairs and clusters. Only report if greater than normal flora present. Quantitate and enter the code gram positive cocci in pairs and clusters – Impression Staphylococcus.
- Gram negative rods may be seen. Quantitate and report only if they are the predominate organism, in small numbers they can be included as normal flora.

### 4. Wound Specimens

- Review the slide using 100x, oil immersion. Quantitate and report the following:
  - WBC'S (presence or absence)
  - Epithelial cells (if present)
  - RBC's (if present)
  - Yeast and Bacteria

- Look for gram positive cocci in chains and add interpretation of Gram positive cocci (Impression of Streptococci) or gram positive cocci in clusters (Impression of Staph) or GPC.
- Review slide for gram negative rods and quantitate.
- Review slide for gram positive rods and look like for organisms that appear like Chinese letters or stacked up. These are diphtheroids. Quantitate and enter gram positive bacilli resembling Diphtheroids. If the organisms do not have a diphtheroid appearance report as gram positive rods.
- If no organisms are present, report No organisms seen.

## 5. CSF/Body Fluids

- Review the slide using 100x, oil immersion.
- Quantitate and report out the presence or absence of WBC's and RBC'S. If no WBC's are seen, report NO WBC, if no RBC's are seen, report NO RBC.
- Scan a large area of the slide to look for bacteria, if no bacteria are present, report NOS. Be careful to not interpret artifacts such as stain debris as bacteria, compare with known slides if needed.
- If you think bacteria are present, it may be helpful to review the hematology or chemistry results. The WBC response in bacterial infections or meningitis is usually polys. A viral infection is usually lymphs. CSF glucose is usually depressed in bacterial or TB meningitis cases and normal in other cases. CSF Protein can be elevated. In partially treated cases of bacterial meningitis, cellular and chemical findings and the recovery of bacteria may be altered. Gram positive organisms may appear to be gram negative due to the antibiotic reaction on the cell wall.
- Streptococci and gram negative cocci can often look rod shaped on CSF direct smears. If in doubt, ask a Microbiologist or a pathologist to review the smear.
- All positive CSF smears are a critical value and must be phoned to the provider immediately.
- Common organisms found in the CSF are Neisseria meningitidis, Streptococcus pneumoniae, Strep Group B, Listeria and Haemophilus.

## 6. Fecal Leukocytes

- Make the smear thin enough so that the smear can be evaluated properly. If the smear is already made, find a thin area to review.
- Look for WBC's on low power, confirm with 100x, quantitate and report.
- In the LIS, report fecal leukocytes present or no fecal leukocytes seen using Result entry.
- When WBC's are present, also look for Campylobacter sp. These are gram negative tiny curved, "bat wing" shaped rods. Quantitate and report Campy organisms present.

## REVIEW AND DOWNTIME

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- All smears performed on the evening and night shifts, and slides from other FHS labs will be reviewed by the Micro day shift staff. If a corrected report is indicated, enter Results Correction under the Actions tab. Add the corrected report. Call the floor and /or physician with the correction. Document the call using the Communications Log. Fill out a Quality report and give to the dept. manager.
- Slides will be kept in a slide box near the microscope for 1 week and discarded in a sharps container.
- A downtime form can be used when the computer is unavailable. This form can be found in the downtime book.
- All critical results will be called to the floor or physician and documented in the computer according to policy.
- Stat requests will be performed within 30 minutes of receipt of the specimen into Microbiology.

## LIMITATIONS

- Slides under-decolorized will appear blue making it difficult to distinguish blue from red. May be able to restain the slide.
- Slides over-decolorized will appear pink and washed out making it difficult to see and distinguish the types of organisms present. A second slide should be stained.
- Decolorizer has to be the correct mixture, half acetone, half ethanol. Decolorizer that is made incorrectly will result in under or over decolorized slides.
- Gram stain reagents can cause artifacts that may be confused with bacteria. Crystal violet can precipitate and look like gram positive cocci. Artifacts can look like gram negative cocci as well. Look for consistency in size and shape. Artifacts usually are not consistent and range in different sizes.

## REFERENCE

1. Murray, Patrick Manual of Clinical Microbiology, 8th ed. American Society of Microbiology, 2003.
2. Koneman, Elmer Color Atlas and Textbook of Diagnostic Microbiology. J. B. Lippincott, 1983.
3. Marler, Linda Direct Smear Atlas. Lippincott Williams and Wilkins, 2001