UPMC Hanover Laboratory

Subject: Gram Stain, Slide Staining and Interpretation	

PURPOSE:

This document describes the procedure for staining and interpretation of Gram stained slides.

SCOPE:

This policy applies to Hanover Hospital Laboratory

PRINCIPLE:

Bacteria stain gram positive or negative due to differences in the make up of the cell wall. During the process of the stain, Gram positive bacteria will retain the crystal violet/ iodine complex of the initial stain, appearing purple when viewed on the slide. After application of a decolorizer, gram negative bacteria do not retain the crystal violet/iodine complex and take up the safranin counterstain, appearing red (pink). when viewed on the slide.

PREPARING AND STAINING THE SLIDE

SPECIMEN:

A Gram stain should not be performed on the following specimen types;

Urines
Cystic fibrosis sputum
Cath tips
Stools
Throat
Nasal

REAGENTS:

Crystal violet
Stabilized iodine
Acetone/Alcohol decolorizer
Gram Safranin
Microscope Slides
Quality Control slides

QUALITY CONTROL:

Quality control is performed weekly using pre made slides. Slides are prepared using *Staphylococus aureus* and *E. coli.*

The QC slide is stained using the same procedure that is used for patient samples

Results are recorded on the Gram stain QC chart.

PROCEDURE:

Preparing the slide

1. For Gram stains of specimens, apply a thin layer of specimen to the side. If the specimen is tenacious, use a second slide pressed against the first one to spread out the specimen.

NOTE: Slides prepared directly from patient specimens must be kept under the hood until completely dry.

2. For colonies, apply a drop of saline or water to the slide and emulsify a portion of the colony in the liquid
3. Slides may be placed on a slide warmer under the hood to dry. The temperature of the slide warmer should NOT exceed 50°C.

Performing the Gram stain

1. Fix the slide using methanol or heat fix.
2. Flood the slide with crystal violet and allow it to stain for a minimum of 15 seconds.
3. Rinse the slide with tap water, holding at an angle to allow run off. Do not aim water directly at the area of the slide that contains the specimen.
4. Flood the slide with Gram's iodine and allow it to stain for a minimum of 15 seconds.
5. Rinse the slide with tap water, holding at an angle to allow run off. Do not aim water directly at the area of the slide that contains the specimen.
6. Flood the slide with the Acetone alcohol decolorizer and immediately rinse. Hold slide at an angle to allow run off. CAUTION: OVER DECOLORIZATION OF THE SLIDE MAY CAUSE RESULT IN ERRONEOUS GRAM STAIN RESULTS.
7. Flood the slide with safranin and allow it to stain for a minimum of 15 seconds.
8. Rinse the slide with tap water, holding at an angle to allow run off. Do not aim water directly at the area of the slide that contains the specimen.
9. Allow the slide to dry in rack or dry with bibulous paper. DO NOT DRY SLIDES CONTAINING CLINICAL SPECIMENS WITH A PAPER TOWEL. Small numbers of organisms present may be removed.

INTERPRETATION:

1. Under low power (10X) evaluate the quality of the stained slide.
   - A properly stained slide should have a clear or pink background.
   - There should be little stain precipitate present. If a large amount is on the slide, the stain should be repeated.
   - White blood cells should exhibit pale pink cytoplasm.
2. Evaluate the number of white blood cells (polymorphonuclear) and epithelial cells present under low power (10X). Report as indicated in the table that follows.

Table 1

Number of cells seen per Low Power Field (10x)	***Report***
<1	Rare
1-9	Few
10-25	Moderate
>25	Many

3. Under High Power (100X oil immersion), note the number, gram reaction and morphology of each microorganism seen. Report organism numbers as indicated in the table that follows.

Table 2

Number of Organisms Seen per Oil Immersion Field (100x)	***Report***
<1	Rare
1-5	Few
6-30	Moderate
>30	Many

4. After the slide is evaluated, report the results as follows:

- Number and presence or absence of white blood cells (PMNs). See table 1.
- Number of epithelial cells if present. See Table 1.
- Number and gram stain characteristics of bacteria or other organisms present. See Table 2.
- Morphology of bacteria or other organisms present. See reporting conventions in Table 3.

Table 3

Gram positive cocci	Singles, pairs, chains, diplococci, groups,clusters, tetrads
Gram positive rods	Large, small, with spores, branching, pairs, chains, palisades
Gram negative cocci	Diplococci (if indicated)
*Gram negative rods	Thin, pointed, tiny, coccobacillus
Yeast	Budding, hyphae (if indicated)
Fungi	Note as being seen on smear.

*Enteric like Gram negative rods may be reported as "Gram negative rods" without further size description.

SPECIAL CONSIDERATIONS:

CSF Specimens

All CSF gram stains are considered STAT. The expected turnaround time is 60 minutes from receipt of the specimen in the laboratory.

If any microorganisms are observed on the gram stain of a CSF specimen, the results must be called to the patient's care provider immediately. This is a critical result. The name of the provider who took the report and the date must be documented in EPIC.

Most CSF specimens from patients with meningitis will also contain inflammatory cells.

Sputum and Tracheal Specimens, Screening

All sputum specimens must be screened for quality. Refer to the table that follows for specimen criteria.

Number of WBCs (PMNs) per low power field	Number of Epithelial Cells			
	None	**Rare or Few**	**Moderate**	**Many**
None	Process	Reject	Reject	Reject
Rare or Few	Process	Reject	Reject	Reject
Moderate	Process	Process	Reject	Reject
Many	Process	Process	Process	Reject

Concentrated Gram Stain (Cytospin)

Add the appropriate EPIC code, to all specimens concentrated for Gram stain.

Review of Second and Third Shift Gram Slides

Gram stains read and reported by second and third shift are reviewed by first shift microbiology staff.

REFERENCES:

UPMC Laboratory Services Center, Manual Staining of Direct Gram Stains. 2023.

UPMC Laboratory Sesrvices Center, Reading of Gram Stained Smears. 2023.

Jorgenson JH et al. Manual of Clinical Microbiology. 11th ed. ASM Press. Washington DC. 2015.

Leber, Amy, Editor. Clinical Microbiology Handbook. 4th edition. 2016.