

SDM GSt SDM Gram Staining

Learning Objectives

The student will

- Use aseptic techniques
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques when making observations.
- Use the bright field light microscope to view microbes under oil immersion, make accurate observations and appropriate interpretations and store the microscope according to lab procedures.
- Properly prepare a bacterial smear for accurate staining and describe the chemical basis for simple staining and negative staining.
- Accurately perform a Gram stain and describe the molecular basis for the technique.

Background/Theory

In the exercises on Selective and Differential Media, you will notice that two of the media you will be using employ selective agents that inhibit organism growth based on their grouping with Gram positive organism or with Gram negative organisms. Therefore, to properly interpret the results of those exercises, you will need to know the Gram staining characteristic of each of the organisms.

Experiment/Exercise

Materials per student

2 microscope slides

Gram staining reagents and supplies

Cultures

Fresh overnight broth/plate cultures

Staphylococcus aureus

Pseudomonas aeruginosa

Streptococcus agalactiae (Risk Group 2, BSL-2 precautions)

Enterococcus faecalis?

Streptococcus pneumoniae (Risk Group 2, BSL-2 precautions)

Mixed culture control *E. coli* and *S. epidermidis*

Procedure

1. Notice that you are using organisms from Risk Group 2 this week! Use extra caution.
2. Each person will stain one organism from the list and then share their results with other students around them. With your partner and the students near you, discuss who will stain which organism.
3. Each person will make a slide with two smears, the mixed culture control smear and the organism of unknown Gram staining characteristic. You may make a second identical slide for use as a backup if your first Gram stain does not turn out correctly. (DO NOT stain both slides at the same time!)

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4. After heat fixing both slides, Gram stain ONE of them. Verify that your techniques is good by observing cells under oil immersion on the mixed control side first. Get the instructor's initials on your data table.
5. Once you know your technique is correct, find the unknown cells on the other smear of the same slide under oil immersion.
6. Draw a representative group of cells according to the guidelines described in SSt. Indicate morphology and arrangement using the scientific terms in SSt and record the Gram reaction.
7. Obtain the morphologies, arrangements and Gram reactions of the other unknown organisms from neighboring students.
8. You should already know this information for *S. epidermidis* and *E. coli*. Add it to the table.
9. Be sure to write out the full scientific name in the correct form for each organism.

Lab Report: SDM Gram Stain

Name _____

Lab Section _____

Data and Observations

Organism	color	Gram rxn	Morphology	Arrangement	instr initials
<i>E. coli</i>					
<i>Staphylococcus epidermidis</i>					

Your organism _____

Drawing

