

Microbiology for the Health Sciences Laboratory

Lab Session 4 - Staining Techniques

Introduction

Microscopes magnify, and with proper lenses and illumination for resolution, one can almost visualize microorganisms using a good microscope. However, for proper visualization, one needs contrast. Contrast is achieved in brightfield microscopy by staining the specimen. General staining can be achieved with a simple stain, but special stains and techniques are required to stain microbial structures or to separate microorganisms based on their chemical differences. The purpose of this lab is to learn how to use some common staining techniques to aid visualization of microorganisms.

A. Review the following for reference:

1. Text: Chapter 3 - Pages 46-51.
2. Pay particular attention to the following:
 - Techniques used in the preparation of Smears
 - Techniques used in performing Differential Stains
 - Techniques used in performing Structural Stains
 - Structural anatomy of procaryotes

B. Do the following during lab:

1. Gram Stains (Work alone)
 - a. Use 1 microscope slide and your "chosen bug" from last week (Part B1a & B2):
Make 3 smears - 1 each from the broth, the slant and the streak plate.
 - b. Use 1 microscope slide and the streak plate from your throat swab from last week (B1c):
Make 3 smears - 1 each from 3 colonies of microorganisms that have a distinctly different morphological appearance. (Note: You can also use colonies from someone else's streak plate if yours is a mess or you can't identify 3 different m.o.'s, or if there is no growth.)
 - c. Use 1 microscope slide and the BHIA slant(s) of your second mo from last week (Part B3):
Make 1 smear
 - d. Optional: Use 1 microscope slide and the streak plate from the environmental swab (if you did this):
Make 3 smears - 1 each from 3 different mo's
 - e. Do a gram stain on each of the 3-4 slides.
Observe at 1000x (oil) and describe the following:
Size, Shape, Color and Arrangement, as well as any additional observations.
2. Observe ONE gram stain of each of the other m.o.'s (in your group) and describe it.
3. Observe the demonstrations slides and describe the structures.
4. Reisolation:
Reisolate **one** colony from your throat swab streak plate and transfer to a new TSA plate using the streak plate technique. Next week, after you have isolated your mo, you will Gram Stain an isolated colony and then transfer an inoculum to a TSB tube. We will use your isolated and gram stained mo in 2 weeks!

C. Techniques:

1. Basic Staining Procedures:

Gram Stain

- Prepare smear and air dry. (Don't use excessive heat to dry.)
- Heat fix. (Correct temperature = very warm, not too hot, to the touch.)
- Stain with Crystal Violet for 45-60 sec and then rinse with water until color stops.
- Cover with Iodine for 45-60 sec and then rinse with water.
- Rinse with Alcohol until color stops running; 3-4 droppers of alcohol is usually sufficient; and then rinse with water.
- Stain with Safranin for 30-40 sec and then rinse with water until color stops.
- Blot dry with bibulous paper and observe.

2. Basic Technique to Prepare a Smear:

3. Basic Technique to Prepare a Stain (Gram Stain)

D. Results:

Record your observations in your notebook: Size, shape, organization, structures and stain reactions.

B1a. Gram Stains: Chosen Microorganism. (either *S. epidermidis*, *B. subtilis* or *E. coli*)

Slant

Broth

Streak Plate

B1b. Throat Swab: Isolated colonies

#1

#2

#3

B1c. Other Microorganisms:

C. xerosis

S. salivarius

Ps. fluorescens

S. cerevisiae

N. sicca

B1c. Optional: Environmental Swab: Isolated Colonies

#1

#2

#3

B2. Other Microorganisms (from your group):

#1

#2

#3

B4. Demonstration Slides:

Spores

Capsules

Flagella

Fat Bodies

Nucleoplasm

Acid Fast

E. Discussion and Conclusions: