

General Microbiology Lab

Lab Session 3 - 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. Lab Manual: Exercise #3 - Pages 23 to 25. Note especially Fig. 3.1.
Exercise #5 - Pages 33 to 35. Note especially Fig. 5.1.
Exercise #6 - Pages 39 to 41. We will use a technique that does not require heat!
Exercise #7 - Pages 45 to 47. We will use some very simple techniques on already prepared smears!
2. Text: Chapter 2 - Pages 25 to 28.
3. 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

B. Do the following during lab:

1. Gram Stains: (Work with your group and do the following)
 - a. Use 4 microscope slides and your mo's (*Bacillus sp*, *Staphylococcus sp*, *E. coli* & *Neisseria sp*) from last week:
Make 3 smears - 1 each from the broth, the slant and the streak plate for each mo.
 - b. Use 1 microscope slide and the streak plate from your throat swab:
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.
 - d. Use 4 microscope slides and the slants of the second mo's (*Saccharomyces sp*, *Corynebacteria sp*, *Streptococcus sp* and *Pseudomonas sp*) :
Make 1 smear of each.
 - e. Do a gram stain on each of the slides.
Observe at 1000x (oil) and describe the following:
Size (relative size!), Shape, Color and Arrangement, and any additional observations.
2. Observe and describe the gram stain (from streak plate, slant & broth) for ONE of the organisms in B.1.a.
Observe a gram stain for each of the other seven m.o.'s (in B.1.a and B.1.d).
You do not need to observe or describe the throat mo's from other students.
- 3.
4. Observe the demonstration slides and describe the structures.
5. Do the following:
 - Reisolate (i.e. from your streak plate) one mo from your throat. Use a new TSA plate and do a streak plate. Make sure you get good isolated colonies.
 - Later you will gram stain one isolated colony and transfer it to a TS broth tube. We will use this in 2 weeks.

Techniques:

1. Basic Staining Procedures for Differential and Structural Stains:

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 (not lens paper!) and observe.

Acid-Fast Stain (Kinyoun's Method)

- Skip

Capsule stain

- Obtain a slide labeled "C"
- Cover the smear for 2-5 minutes with crystal violet.
- After 2-5 minutes allow the stain to drain into the sink.
- Immerse the smear in the beaker of 20% copper sulfate and swirl for 15-30 seconds.
- Rinse well with distilled water, blot with bibulous paper until dry, and observe.

Flagella stain

- Obtain a slide labeled "F"
- Cover the smear with mordant (iodine) for 10 minutes.
- Rinse with water to remove the mordant.
- Cover the smear with fuchsin for 10 minutes.
- Rinse well with distilled water, blot with bibulous paper until dry, and observe.

Endospore stain

- Obtain a slide labeled "E"
- Cover the smear with toluidine blue for 10 minutes.
- Rinse with distilled water, blot with bibulous paper until dry, and observe.

2. Basic Technique to Prepare a Smear:3. Basic Technique to Prepare a Stain (Gram Stain)

1d. Throat Swab: Isolated colonies

#1

#2

#3

4. Demonstration Slides:

Spores

Capsules

Flagella

Fat Bodies

Nucleoplasm

Metachromatic granules

E. Discussion and Conclusions: