

General Microbiology Lab

Lab Session 1 - Microscopy and Microbial Diversity

Introduction

Basic microscopy is a fundamental tool for the microbiologist. However, because our subjects are so small, we not only need to magnify them, we also need to achieve optimum resolution of our specimens. The purpose of this lab is to develop the proficiency necessary to use the brightfield microscope skillfully. While improving our microscopy skills, we will take the opportunity to study the diversity of microscopic organisms in the biosphere.

A. Review the following for reference:

1. Lab manual:
Exercise 1: Pgs 7-10. Exercise 9: Pgs 59-60. Exercises 33 to 36: Pgs 201 to 222.
2. Text: Chapter 2: Pgs 17-25. Chapters 19 - 26: Review only! Note only the basic classification schemes. Chap 35: Pg 861 (for Safety Guidelines).
3. Pay particular attention to the following: Metric units, wavelengths of light, magnification, resolution, components of light microscope (illumination source, condenser, and ocular and objective lenses), and types of light microscope (brightfield, darkfield, phase and fluorescence).
4. Safety Guidelines: You are responsible for following safety precautions as they are presented in lab! You will not be allowed in lab without a lab coat (e.g. an old shirt). The shirt must be left in lab and must not be a pull-over type t-shirt or sweatshirt! Eating and drinking in the lab is strictly prohibited. You can not bring open containers in the lab, including water bottles in your back-pack. You must handle cultures carefully, and report all 'spills' of microorganisms to the lab instructor. You will be given an 'F' for the lab if you purposefully do not follow safety precautions (e.g. drinking or eating in lab). It is an act comparable to cheating on a test. Read in your text Pg 861 in Chap 35.

B. Do the following during lab:

1. Practice using the light microscope: Proper alignment, proper illumination, focusing and use of the oil immersion lens.
2. Observe microorganisms on the prepared slides: Observe, draw, describe, and classify the organisms into their proper kingdoms. Eucaryotes should be observed at 100X or 400X. Use your imagination when describing them. Procaryotes must be observed at 1000X. Descriptions of procaryotes should include: Color, Size (approximate), Shape and Arrangement of cells.
3. Observe the prepared slides set up on the demonstration microscopes: Observe, draw, describe, and classify the organisms into their proper Kingdoms. You **do not** need to adjust the magnification or stage position.
4. Do a throat swab. Follow the demonstration of this technique. Incubate your culture at 35-37 C for 24-48 hours. The large incubator on the right side of the north wall is set at either 35 or 37 C (i.e. body temperature).
5. Do an environmental swab. Choose the inanimate environment of your choice. Incubate your culture at 28-30 C for 48-72 hours. The incubator on the left is set at 28 or 30 C (i.e. very warm room temperature).

C. Results

1. Record the observations and classifications on the prepared slides in your notebook.
2. Consider organizing your notebook (a small 3-ring binder?) as follows:
 - Lab handout (with notations and directions),
 - Data (carefully organized) in tables or charts,
 - Discussion (a summary of what you did and thoughtful commentary on what it means)

D. Techniques:

1. Microscopy: To align the microscope for optimum resolution:
 - a. Turn on the light source (Main switch).
 - b. Place a slide on the stage (Specimen holder).
 - c. Select the 10X objective.
 - d. Adjust the intensity of light with the sliding voltage control lever.
 - e. Use the coarse, and then the fine focus knobs to focus the specimen.
 - f. Close down the sub-stage iris diaphragm (Field iris diaphragm).
 - g. Move the condenser up or down to focus the light on the specimen. (Condenser height adjustment knob).
 - h. Use the condenser centering knobs to move the beam of light into the center of the field.
 - i. Open the sub-stage iris diaphragm to just fill the field of view with illumination.
 - j. Adjust the condenser Aperture iris diaphragm ring) to match the numerical aperture of the objective.
 - k. Adjust the eyepieces (Diopter adjustment ring) to match your particular vision.
 - l. Technically, you must repeat steps 'd' through 'j' every time you change objectives.

2. Microscopy: To change magnification:
 - a. Focus on a specimen using the 10X objective.
 - b. Move the 40X objective into position.
 - c. Use only the fine focus knob to adjust proper focus.

3. Microscopy: To use oil immersion lens:
 - a. Focus on the specimen using the 10X objective, and then the 40X objective.
 - b. Place a drop of oil on the slide and move the 100X objective into position.
 - c. Use only the fine focus knob to adjust proper focus.
 - d. When finished, clean the objective using lens paper.
 - e. Permanent slides can be cleaned with tissue paper.

4. Culture Techniques: Handling and labeling of culture plates:
 - a. The instructor will demonstrate how to hold and inoculate a culture plate. Although it may seem difficult at first, it is important to improve your dexterity so that you can routinely inoculate culture plates, while minimizing the chance of contamination.
 - b. Label culture plates, on the bottom, with a permanent marker, with the following information: Your name, the date, the type of media, purpose of the plate, and the type of sample or culture, and any other essential information.

5. Culture Techniques: Incubation of culture plates:
 - a. Always incubate the plate at the proper temperature.
 - b. Plates are generally incubated 'upside down' (to retain moisture).
 - c. Plates are generally removed from the incubator in 24-72 hours.

Results: Prepared Slides

1. Fungi. (Note: There are three different species of fungi on the slide. Draw and describe each at 100x.)

Kingdom:

2. Diatoms. (Note: There are many types of diatoms present. Draw and describe several types at 100x.)

Kingdom:

3. *Volvox* sp. (Note: Draw and describe at 100x.)

Kingdom:

4. *Spirogyra* sp. (Note: This organism is in the process of conjugation. Draw and describe at 100x.)

Kingdom:

5. *Spirillum* sp. (Note: This organism is small but has a precise anatomy. Draw and describe at 400x.)

Kingdom:

6. *Micrococcus* or *Gaffkya* or *Streptococcus* sp. (Draw and describe at 1000x: Size, shape, color and arrangement.)
Kingdom:

7. *Klebsiella* sp. (Draw and describe at 1000x: Size, shape, color and arrangement.)
Kingdom:

8. *Neisseria* sp. (Draw and describe at 1000x: Size, shape, color and arrangement.)
Kingdom:

9. *Corynebacterium* sp. (Draw and describe at 1000x: Size, shape, color and arrangement.)
Kingdom:

10. *Salmonella* or *Hemophilus* sp. (Draw and describe at 1000x: Size, shape, color and arrangement.)
Kingdom:

Results: Demonstration Slides

1. Bacteria-Yeast-Blood (Note: Observe at 1000x, draw all three cell types and comment on size.)

Kingdoms:

2. *Giardia lamblia* (trophozoite stage):

Kingdom:

3. *Giardia lamblia* (cyst stage).

4. *Tricinella spiralis*

Kingdom:

5. *Trypanosoma gambiense*:

Kingdom:

6. Liver flukes

E. Discussion and Conclusions: