

Microbiology for the Health Sciences Laboratory

Lab Session 2 - Microbial Diversity

Introduction

It takes time and practice to become proficient at using the light microscope. In addition to developing the skill to align and focus a microscope correctly, it takes insight and patience to be able to make observations on cells that are very small and seemingly have little anatomic diversity. The purpose of this lab is to continue to develop microscopic skills, in particular so that you can observe procaryotic cells. We will also continue to investigate microbial diversity in the biosphere.

A. Review the following for reference:

1. Text:
Chapters 11 & 12 - Review only! Look at the pictures and develop a perspective on microbial diversity.
2. Pay particular attention to the following: General categories of microscopic organisms (i.e. classification of microorganisms into Fungi, Protozoa, Animals and Bacteria).

B. Do the following during lab:

1. Continue: Practice using the light microscope: Proper alignment, proper illumination, focusing and use of the oil immersion lens. Refer to Lab #1, Section C-Techniques.
2. Continue: Observe microorganisms on the prepared slides: Observe, draw, describe, and classify the organisms into their proper kingdoms. Generally, Eucaryotes should be observed at 100X or 400X. Procaryotes must be observed at 1000X. Descriptions of procaryotes should include: Color, Size, 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 37 C for 24-48 hours.
5. Optional: Do an environmental swab. Choose the inanimate environment of your choice. Incubate your culture at 30 C for 48-72 hours.

C. Techniques:

1. Microscopy: Review techniques given in Lab Session #1, Section C.
Use oil liberally when you need to use the oil immersion lens (to get 1000x magnification and optimum resolution). When you are done with a slide, remove the oil from the slide with a Kimwipe. Remove the oil from the objective lens only at the end of lab by using a piece of lens paper.
2. 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 do the techniques correctly 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. Your name (or initials) is required. The rest of the information can be 'coded' (i.e. a complete description of the culture is located in you lab notebook).
3. Culture Techniques: Incubation of culture plates:
 - a. Always incubate the plate at the proper temperature (usually 37 C, 30 C, or 25 C).
Environmental samples are usually incubated at 25 C (i.e. room temperature) and body samples at 37 C (i.e. body temperature).
 - b. Plates are generally incubated 'upside down'. This prevents desiccation of the culture media.
 - c. Plates are generally removed from the incubator in 24-72 hours.

D. Results

1. Record observations on the prepared slides in your notebook.
2. Note in your records the collection of throat and environmental samples.
Record 'where' you took your environmental sample from.

Prepared Slides

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

Kingdom:

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

Kingdom:

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

Kingdom:

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

Kingdom:

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

Demonstration Slides

1. *Treponema pallidum*. (Draw and describe at 1000x: Size, shape, color and arrangement.)

2. *Streptococcus pneumoniae*. (Draw and describe at 1000x: Size, shape, color and arrangement.)

3. Bacteria. (Draw and describe at 1000x: Size, shape, color and arrangement.)

4. Bacteria. (Draw and describe at 1000x: Size, shape, color and arrangement.)

5. Bacteria. (Draw and describe at 1000x: Size, shape, color and arrangement.)

6.

E. Discussion and Conclusions