Chapter 2
Tools of the Laboratory: Methods for the Culturing and Microscopic Analysis of Microorganisms

When you're trying to study microorganisms, you are confronted by some unique problems. First, most habitats (such as the soil, or the human mouth) contain microbes in complex associations, so it is often necessary to separate the species from one another. Second, to maintain and keep track of such small research subjects, microbiologists usually have to grow them under artificial (and thus distorting) conditions. A third difficulty in working with microbes is that they are not visible to the eye. Fourth, microbes are everywhere, and undesirable ones can be introduced into your experiment, causing misleading results.

Many microorganisms can be cultured on artificial media, but some, such as viruses, can only be cultured in living tissues or in cells. Artificial media are classified by their *physical state* (liquid, semisolid, liquefiable solid, or nonliquefiable solid); by their *chemical composition* (defined or complex); or by their *function* (enriched, selective, differential, transport, and so on).

Microbiologists use five basic techniques to manipulate, grow, examine, and characterize microorganisms in the laboratory. These techniques are called the **"Five l's"**: inoculation, incubation, isolation, inspection, and identification. The steps can be viewed as summaries of the laboratory procedures used in microbiology.

Inoculation involves the introduction of a sample into sterile medium. Following *inoculation,* cultures are *incubated* at a specified temperature to encourage growth. *Isolated* colonies that originate from single cells are composed of large numbers of cells piled up together. A culture may be *pure,* containing only one species or type of microorganism; *mixed,* containing two or more known species; or *contaminated,* containing both known and unknown (unwanted) microorganisms.

During *inspection,* the cultures are examined and evaluated macroscopically and microscopically. Microorganisms are *identified* in terms of their macroscopic or immunologic morphology, their microscopic morphology, their biochemical reactions, and their genetic characteristics.

Magnification, resolving power, and contrast all influence the clarity of specimens viewed through the optical microscope. The maximum resolving power of the optical microscope is 200 nm, or 0.2 μm. This resolution is sufficient to see the internal structures of eukaryotes and the morphology of most bacteria.

Of the six types of optical microscopes, four use visible light for illumination: bright-field, dark-field, phase-contrast, and interference microscopes. The fluorescence microscope

uses UV light for illumination. The confocal microscope can use UV light or visible light reflected from specimens. Electron microscopes (EM) use electrons, not light waves, as an illumination source to provide high magnification (5,000× to 1,000,000×) and high resolution (0.5 nm). Specimens viewed through optical microscopes can be either alive or dead, depending on the type of specimen preparation, but all EM specimens must be dead because they are viewed in a vacuum.

Staining of sample is an important technique in microbiology. Stains increase the contrast of specimens and can be designed to differentiate cell shape, structure, and biochemical composition of the specimens being viewed. Negative and positive staining are the two basic types of staining techniques. The Gram stain is an immensely useful differential stain that divides bacteria into two main groups: gram-positive and gram-negative. Some bacteria, such as those that cause tuberculosis, do not fall in either of these categories. The bacteria can be identified with other staining procedures, such as acid-fast staining, endospore staining, and flagellar staining.

Pre-Class Ideas for Chapter 2

Below are suggested activities to assign before covering the material of Chapter 2 in class. The activities are designed to provide opportunities for students to engage with the topics prior to class. Some activities also have students preparing materials that will enable students to teach one another in class.

1. Assign one of the Five I's to groups of students. Have them prepare a mini-lesson to teach the class about their assigned Five I.

2. Provide students a list of different media (TSA, blood agar, mannitol salt, MacConkey, urea broth, TSA broth, birdseed, tomato juice agar, chocolate, etc.). Have students create a chart and for each medium addressing the following: the physical state, whether it is chemically-defined or complex, and the functional type.

3. Have students use simple drawings (in color) to show how organisms appear on the following: general purpose media, selective media, differential media, streak plate, loop dilution, spread plate.

4. Assign groups of students to demonstrate the following to the class using appropriate materials: inoculation of a plate, inoculation of a broth tube, streak plate, loop dilution plate, spread plate, smearing a sample, fixing a sample, hanging drop.

5. Assign the figures located in the chapter to students and have them prepare an explanation to teach the class.

6. In simple drawings (in color), have students create examples of mixed cultures, pure culture, positive stain, negative stain, simple stain, differential stain, endospore stain, capsule stain.

7. Have students write descriptions for various figures within the chapter in their own words.

8. Have students label a diagram of a microscope.

9. Using their own words and simple drawings, have students demonstrate an understanding of magnification, resolution, and contrast.

10. In groups, students create an activity to teach the metric system to classmates.

Activities Associated with Learning Objectives for Chapter 2

Section 2.1 Student Learning Objectives—How to Culture Microorganisms

1. *Explain what the Five I's are and what each step entails.*
2. *Discuss three physical states of media and when each is used.*
3. *Compare and contrast selective and differential media, and give an example of each.*
4. *Provide brief definitions for* defined media *and* complex media.

Lecture Suggestions and Guidelines for Section 2.1

1. The Five I's are critical to microbiology and students will encounter these topics repeatedly throughout the semester. It is helpful to relate each of the Five I's to "real world" scenarios.
2. The classification of media can be confusing to students at first. Students need to understand that media is classified according to its physical state, chemical composition, and function.
3. Introduction of specific media and their role in a microbiology laboratory is aided by presenting the material in a way that relates it to infectious diseases.

In-Class Activities for Section 2.1

1. In a role-play situation, have students walk through the Five I's in the following scenario: a patient presents at doctor's office with severe cough, fever, and sore throat.
2. Provide pictures of cultures growing on identified media. Have students use class time to research growth appearances on a given medium type and give a guess of the type of organisms inoculated on the plates.
3. Bring in examples of different forms of media and allow students to discuss the classification of the media and the purpose of the media.

4. Bringing in appropriate materials, have students demonstrate the following techniques to the class: inoculation of a plate, inoculation of a broth tube, streak plate, loop dilution plate, spread plate, smearing a sample, fixing a sample, hanging drop.

Additional Research Issues for Section 2.1

1. Have students research situations in which an infectious disease was, at first, incorrectly identified. What issues may have contributed to the misidentification?
2. Ask students to research a current infectious disease scenario in the news.
    - Have the students examine how the Five I's of microbiology are being applied in this situation.
    - Ask students to research which of the Five I's may be the most difficult to complete given the situation. Have students provide an explanation for their reasoning.

Critical Thinking Issues for Section 2.1

1. Can the Five I's of microbiology be completed for every disease?
2. Where may potential mistakes be made in regard to the incorporation of the Five I's? How may these mistakes affect our understanding of a disease? What can be done to reduce these errors?
3. Not all microorganisms can be grown on media. What effect does this have on treatment and prevention of these infectious diseases?

Section 2.2 Student Learning Objectives—The Microscope

5. *Convert among the different units of the metric system.*
6. *List and describe the three elements of good microscopy.*
7. *Differentiate between the principles of light microscopy and the principles of electron microscopy.*
8. *Give examples of simple, differential, and special stains.*

Lecture Suggestions and Guidelines for Section 2.2

1. Students will likely have been taught the metric system before, although some may continue struggle with the concepts. Finding a way to help connect students to the metric system may help them begin to grasp the concepts.
2. Emphasis on the three basic principles of microscopy will help students understand the different forms of microscopy.
3. Staining procedures play a large role in microbiology. It is important that students can confidently engage with the terminology associated with staining, in order that the student can apply this knowledge to specific staining procedures and the purpose of such procedures.

In-Class Activities for Section 2.2

1. Hands-on activities for the metric system should be incorporated. These may include student presentations and/or the use of examples of metric devices to measure, weigh, and determine volume.
2. Using a video that shows the “powers of ten” can help students understand the metric system scale.
3. Examples showing resolution, contrast, and magnification in regard to microscopy should be incorporated into the classroom. These may include drawings created by students or figures from the book.
4. Have a microscope (or several) available in the classroom so students can see the microscope components rather than looking at an image of a microscope.
5. Provide a list of different items (human cells, bacteria, protozoa, organelles, viral particles) and have students discuss which form of microscopy would be best suited for viewing these items.
6. Create a discussion chart that outlines how the different forms of microscopy are used to diagnose infectious diseases.
7. Have on hand items typically used in microbial staining procedures so students become familiar with these materials.
8. Have students draw an image of a gram-positive and gram-negative bacterial cell wall and show what each stage of the Gram stain process does to the cell wall.

Additional Research Issues for Section 2.2

1. Research which countries in the world use the metric system and which do not.
2. Research current developments in microscopy. Predict how these developments may be applied to microbiology, specifically infectious diseases.
3. Have students find an image of the same organism using different microscopes and compare the images.
4. Viable but nonculturable (VBNC) bacteria have been discovered in human tissue. What are these bacteria and what role may they play in disease and health?

Critical Thinking Issues for Section 2.2

1. The Gram stain was developed in 1884. Why is this procedure still used today? What (if any) changes have been made to the procedure in modern times?
2. How would the identification and treatment of infectious agents change if all bacteria truly did look and act the “same”?

SmartGrid Bloom’s Level 5 & 6 Activities for Chapter 2

1. **Activity for Question #3:** Using either your book or an internet search, identify what bacterial capsules are and why they are formed. Then consider the types of environments microbes exist in from Chapter 1 and determine the advantages and disadvantages of utilizing energy and nutrient resources in forming the capsule.

2. **Activity for Question #6 & #21:** This chapter focused on one primary stain known as a Gram stain. There are variety of other stains used for identifying different structures in or around the bacterial cell. Research these stains, including their uses and mechanisms of staining. Then, compare and contrast how each stain works. Finally, after reviewing a variety of staining mechanisms, identify common issues that can arise from the staining process and false assumptions that can be made as a result.
3. **Activity for Question #9:** Penicillin has a fascinating history. Research the history of penicillin and compile a time line of major events in penicillin's history through present day.
4. **Activity for Question #12:** Differential media is often a quick way to distinguish between key groups of bacteria. This media often reacts with products produced by the cell. Research different types of differential media and create a list of what these media are used for and what types of bacteria these media can help identify. Finally, using this list, research the cellular products that are being detected and determine their purpose for the cell that produced them.
5. **Activity for Question #15 & #18:** Creative culturing methods are critical to growing bacteria that do not naturally thrive in the same environment we do. Research culture techniques for microbes outside of our normal environmental conditions and prepare a list of these techniques and the types of microbes these techniques are designed to culture.