Chapter 2
Tools of the Laboratory: The Methods for Studying Microorganisms

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× to1,000,000×) 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 they can be designed to differentiate cell shape, structure, and biochemical composition of the specimens being viewed. 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.

Pre-Class Ideas for Chapter 2

Below are suggested activities to assign before covering the material of Chapter Two 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 5 I's to groups of students. Have the student groups teach the class about their assigned 5 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 address the following: the physical state, whether chemically-defined or complex, and the functional type.

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

4. Assign student groups to demonstrate 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 following figures to student(s) and have them prepare an explanation to teach the class: Figure 2.2, Figure 2.3, Figure 2.5, Figure 2.6, Figure 2.7, and Figure 2.9.

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 Figures 2.17, 2.18, 2.19 in their own words.

8. Students label a diagram of a microscope.

9. Using their own words and simple drawings, 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

2.1 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 5 I's are critical to microbiology and students will encounter these topics repeatedly throughout the semester. It is helpful to relate each of the 5 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 5 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 5 I's of microbiology are being applied in this situation.
    - Ask students to research which of the 5 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 5 I's of microbiology be completed for every disease?
2. Where may potential mistakes be made in regards to the incorporation of the 5 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 fact have on treatment and prevention of these infectious diseases?

2.2 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.
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. Examples showing resolution, contrast, and magnification in regards to microscopy should be incorporated into the classroom. These may include drawings created by students or figures from the book.
3. 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.
4. 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.
5. Create a discussion chart that outlines how the different forms of microscopy are used to diagnosis infectious diseases.
6. Have on hand items typically used in microbial staining procedures so students become familiar with these materials.

Additional Research Issues for Section 2.2

1. Research countries in the world in regards to which 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. 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”?