

PREFACE

Take interest, I implore you, in those sacred dwellings which one designates by the expressive term: laboratories. Demand that they be multiplied, that they be adorned. These are the temples of the future—temples of well-being and of happiness. There it is that humanity grows greater, stronger, better.

Louis Pasteur

(French chemist, founder of microbiology, 1822–1895)

There are many excellent microbiology laboratory manuals on the market and many others that are called “in-house” productions because they are written for a microbiology course at a particular school. Why another microbiology manual? The answer is straightforward. Many instructors want a manual that is directly correlated with a specific textbook. As a result, this laboratory manual was designed and written to be used in conjunction with the textbook *Microbiology*, fifth edition, by Lansing M. Prescott, John P. Harley, and Donald A. Klein; however, it can be used with other textbooks with slight adaptation.

Since this manual correlates many of the microbiological concepts in the textbook with the various exercises, comprehensive introductory material is not given at the beginning of each exercise. Instead, just enough specific explanation is given to complement, augment, reinforce, and enhance what is in the textbook. We feel that time allocation is an important aspect of any microbiology course. Students should not be required to reread in the laboratory manual an in-depth presentation of material that has already been covered satisfactorily in the textbook.

Each exercise has been designed to be modular and short. This will allow the instructor to pick and choose only those exercises or parts of exercises that are applicable to a specific course. Several exercises usually can be completed in a two- or three-hour laboratory period. The exercises have also been designed to use commonly available equipment, with the least expense involved, and to be completed in the shortest possible time period.

Considering the above parameters, the purpose of this laboratory manual is to guide students through a process of development of microbiological technique, experimentation, interpretation of data, and discovery

in a manner that will complement the textbook and make the study of microbiology both exciting and challenging. According to an old Chinese proverb:

Tell me and I will forget.

Show me and I might remember.

Involve me and I will understand.

These words convey our basic philosophy that it is experiences in the microbiology laboratory and the scientific method that help develop students' critical thinking and creativity and that increase their appreciation of the mechanisms by which microbiologists analyze information. The laboratory accomplishes this by having students become intensely and personally involved in the knowledge they acquire.

The array of exercises was chosen to illustrate the basic concepts of general microbiology as a whole and of the individual applied fields. The protocols vary in content and complexity, providing the instructor with flexibility to mold the laboratory syllabus to the particular needs of the students, available time and equipment, and confines and scope of the course. Furthermore, it provides a wide spectrum of individual exercises suitable for students in elementary and advanced general microbiology as well as those in various allied health programs.

In 1997, the American Society for Microbiology, through its Office of Education and Training, adopted a Laboratory Core Curriculum representing themes and topics considered essential to teach in every introductory microbiology laboratory, regardless of its emphasis. An instructor might add items appropriate to allied health, applied, environmental, or majors microbiology courses.

The Laboratory Core is not meant to be a syllabus or outline. The core themes and topics are meant to frame objectives to be met somewhere within the introductory microbiology laboratory. Depending on the

specific emphasis of the course, a single lab session could meet multiple core objectives, focus on one objective, or emphasize a topic that is not in the lab core but is important to that particular course.

Laboratory Skills

A student successfully completing basic microbiology will demonstrate the ability to

- 1. Use a bright-field light microscope** to view and interpret slides, including
 - a. correctly setting up and focusing the microscope
 - b. proper handling, cleaning and storage of the microscope
 - c. correct use of all lenses
 - d. recording microscopic observations
- 2. Properly prepare slides** for microbiological examination, including
 - a. cleaning and disposal of slides
 - b. preparing smears from solid and liquid cultures
 - c. performing wet-mount and/or hanging drop preparations
 - d. performing Gram stains
- 3. Properly use aseptic techniques** for the transfer and handling of microorganisms and instruments, including
 - a. sterilizing and maintaining sterility of transfer instruments
 - b. performing aseptic transfer
 - c. obtaining microbial samples
- 4. Use appropriate microbiological media and test systems,** including
 - a. isolating colonies and/or plaques
 - b. maintaining pure cultures
 - c. using biochemical test media
 - d. accurately recording macroscopic observations
- 5. Estimate the number of microorganisms in a sample** using serial dilution techniques, including
 - a. correctly choosing and using pipettes and pipetting devices
 - b. correctly spreading diluted samples for counting
 - c. estimating appropriate dilutions

- d. extrapolating plate counts to obtain correct CFU or PFU in the starting sample

6. Use standard microbiology laboratory equipment correctly, including

- a. using the standard metric system for weights, lengths, diameters, and volumes
- b. lighting and adjusting a laboratory burner
- c. using an incubator

Laboratory Thinking Skills

A student successfully completing basic microbiology will demonstrate an increased skill level in

- 1. Cognitive processes,** including
 - a. formulating a clear, answerable question
 - b. developing a testable hypothesis
 - c. predicting expected results
 - d. following an experimental protocol
- 2. Analysis skills,** including
 - a. collecting and organizing data in a systematic fashion
 - b. presenting data in an appropriate form (graphs, tables, figures, or descriptive paragraphs)
 - c. assessing the validity of the data (including integrity and significance)
 - d. drawing appropriate conclusions based on the results
- 3. Communications skills,** including
 - a. discussing and presenting laboratory results or findings in the laboratory
- 4. Interpersonal and citizenry skills,** including
 - a. working effectively in groups or teams so that the task, results, and analysis are shared
 - b. effectively managing time and tasks to be done simultaneously, by individuals and within a group
 - c. integrating knowledge and making informed judgments about microbiology in everyday life

Laboratories typically supplement and integrate closely with the lecture content in ways that are unique to each instructor. Consequently, the laboratory content that is considered essential for laboratory work by one instructor may be covered in lecture portion of the course by another instructor, making it difficult to define specific top-

ics that should be integral in all microbiology laboratories. As a result, the ASM Laboratory Core Curriculum Committee developed themes, which are broadly based and will enable instructors to have the flexibility to use a wide variety of laboratories to meet the suggested core.

A student successfully completing basic microbiology will demonstrate mastery of the basic principles of the following themes and complete laboratory activities that focus on one or more of the topics under each theme.

Theme 1. Integrating themes—impact of microorganisms on the biosphere and humans; microbial diversity

Theme 2. Microbial cell biology, including cell structure and function, growth and division, and metabolism

Theme 3. Microbial genetics, including mutations

Theme 4. Interactions of microorganisms with hosts (humans, other animals, plants), including pathogenicity mechanisms and antimicrobial agents

In order to meet the above themes, topics, and skills (The American Society for Microbiology Laboratory Core Curriculum), this manual consists of 66 exercises arranged into 11 parts covering the following basic topics:

PART ONE, Microscopic Techniques, introduces the students to the proper use and care of the different types of microscopes used in the microbiology laboratory for the study of microorganisms.

PART TWO, Bacterial Morphology and Staining, presents the basic procedures for visualization and differentiation of microorganisms based on cell form and various structures.

PART THREE, Basic Laboratory and Culture Techniques, acquaints students with proper laboratory procedures in preparing microbiological media and in culture techniques that are used in isolating microorganisms.

PART FOUR, Biochemical Activities of Bacteria, introduces some of the biochemical activities that may be used in characterizing and identifying bacteria.

PART FIVE, Rapid Multitest Systems, acquaints students with some of the multitest systems that can be used to identify bacteria.

PART SIX, Unknown Identification, contains two exercises that guide students through the use of

Bergey's Manual of Systematic Bacteriology in the identification of unknown bacteria.

PART SEVEN, Environmental Factors Affecting Growth of Microorganisms, acquaints students with some of the various physical and chemical agents that affect microbial growth.

PART EIGHT, Environmental and Food Microbiology, is concerned with the environmental aspects of water, milk, and food.

PART NINE, Medical Microbiology, presents an overview of some pathogenic microorganisms, and acquaints students with basic procedures used in isolation and identification of pathogens from infected hosts, including those from the student's own body.

PART TEN, Survey of Selected Eucaryotic Microorganisms, presents an overview that is intended to help students appreciate the morphology, taxonomy, and biology of the fungi.

PART ELEVEN, Microbial Genetics and Genomics, presents six experiments designed to illustrate the general principles of bacterial genetics and genomics.

The format of each exercise in this manual is intended to promote learning and mastery in the shortest possible time. To this end, each experiment is designed as follows:

Safety Considerations

This laboratory manual endeavors to include many of the safety precautionary measures established by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia; the Occupational Safety and Health Administration (OSHA); and the Environmental Protection Agency (EPA). Efforts are made to instruct the student on safety, and all exercises will contain precautionary procedures that these agencies are enforcing in hospitals, nursing homes, commercial laboratories, and industry. A **safety considerations box** is included for each exercise to help both the instructor and student prepare themselves for the possibility of accidents.

Both the instructor and student should keep in mind at all times that most technical programs, such as a microbiology laboratory, carry some measure of associated risk. The microbiology laboratory is a place where infectious microorganisms are handled, examined, and studied with safety and effectiveness. However, any of the microorganisms we work with

may be pathogenic in an immunocompromised person. Therefore, rather than modifying the objectives in this laboratory manual to avoid any risk, the authors propose that instructors and students implement the Centers for Disease Control and Prevention (CDC) principles of biosafety throughout. One way we propose is to simply modify the "Universal Precautions" (see pp. xiii–xiv) so the wording is appropriate for the classroom by simply changing "laboratory worker" to "student." In addition, a written safety policy consistent with CDC guidelines and adopted by your institution's governing body will protect you, your institution, and the students. As in any laboratory, safety should be a major part of the curriculum. Students should be required to demonstrate their knowledge of safety before they begin each laboratory exercise.

Materials per Student or Group of Students

To aid in the preparation of all exercises, each procedure contains a list of the required cultures with American Type Culture Collection catalog numbers (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 29852–1776; www.ATCC.org; 703-365-2700), media, reagents, and other equipment necessary to complete the exercise in the allocated lab time either per student or group of students. Appendixes H and I provide recipes for reagents, stains, and culture media. Appendix J describes the maintenance of microorganisms and supply sources.

Learning Objectives

Each exercise has a set of learning objectives that define the specific goals of the laboratory session. It is to the student's advantage to read through this list before coming to class. In like manner, these objectives should be given special attention during the laboratory exercise. Upon conscientious completion of the exercise, the student should be able to meet all of the objectives for that exercise. Before leaving the class, students should check the objectives once again to see that they can master them. If problems arise, consult the instructor.

Suggested Reading in Textbook

These cross-references have been designed to save the student's time. By referring the student to sections, paragraphs, tables, charts, figures, and boxes within the textbook, unnecessary duplication is avoided.

Pronunciation Guide

This section contains the phonetic pronunciations for all organisms used in the exercise. If students take the time to sound out new and unfamiliar terms and say them aloud several times, they will learn to use the vocabulary of microbiologists.

Why Are the Above Bacteria, Slides, or Other Microorganisms Used in This Experiment?

The authors have chosen specific viruses, bacteria, fungi, protozoa, algae, and various prepared slides for each exercise. This microbial material has been selected based on cost, ease of growth, availability, reliability, and most importantly, the ability to produce the desired experimental results. In order to communicate these guidelines to the student, this section explains why the authors have chosen the microbial material being used and also gives additional biochemical, morphological, and taxonomic information about the microorganism(s) that the student should find helpful when performing the experiment.

Medical Application

Many students using this laboratory manual are either in one of the allied health disciplines, such as nursing, or in a preprofessional program such as premed, pre-dent, or prevet and need to know the clinical relevance of each exercise performed. To satisfy this need, a *Medical Application* section is included for some of the medically oriented exercises. Medical applications are described for most clinical procedures as a specific application of the purpose of the exercise. For example, a procedure can be used for the identification of a particular microorganism or used in combination with other exercises in a diagnosis. For these exercises, some important pathogens with their diseases and their need for the test being performed in the exercise are listed.

Principles

This section contains a brief discussion of the microbiological principles, concepts, and techniques that underlie the experimental procedures being performed in the exercise.

Procedure

Explicit instructions are augmented by diagrams to aid students in executing the experiment as well as interpreting the results. Where applicable, actual results are shown so that the student can see what should be obtained.

Hints and Precautions

Additional information on what to watch out for, what can go wrong, and helpful tidbits to make the experiment work properly are presented in accompanying boxes.

Laboratory Report

Various pedagogical techniques are used for recording the obtained results. This part of the exercise can be turned in to the instructor for checking or grading.

Review Questions

Review questions are located at the end of each laboratory report. These were written so that students can test their understanding of the concepts and techniques presented in each exercise.

Dilution Ratios Used in This Manual

According to the *American Society for Microbiology Style Manual*, dilution ratios may be reported with either colons (:) or shills (/), but note there is a difference between them. A shill indicates the ratio of a part to a whole; e.g., $\frac{1}{2}$ means 1 of 2 parts, with a total of 2 parts. A colon indicates the ratio of 1 part to 2 parts, with a total of 3 parts. Thus, $\frac{1}{2}$ equals 1:1, but 1:2 equals $\frac{1}{3}$.

Dilution Problems

Since dilution problems are such an integral part of any microbiology course, *Appendix A* gives an overview of

the different types of dilution. This includes a variety of practice problems. Answers are provided.

Instructor's Guide

An instructor's guide has been prepared for the laboratory manual and is available on our web site at www.mhhe.com/prescott5. This guide provides answers to the questions in this manual.

Finally, it is our hope that this manual will serve as a vehicle to (1) introduce the complexity and diversity of microorganisms and their relationships to one another; (2) provide a solid foundation for further study for those electing a career in science; and (3) convey something of the meaning, scope, and excitement of microbiology as a significant perspective from which to view the world.

We appreciate the many comments offered to us over the years by both faculty and students. In our desire to continue to improve this laboratory manual, we invite constructive comments from those using it. Please contact us through the Cell and Molecular Biology Editor, McGraw-Hill Publishers (www.mhhe.com/prescott5).

John P. Harley
Lansing M. Prescott