Preface

Laboratory Experiments in Microbiology, Eighth Edition, is designed to supplement any nonmajors microbiology textbook. Co-authored by Christine L. Case, this manual is an ideal companion to *Microbiology: An Introduction*, Ninth Edition, by Tortora, Funke, and Case. Our goal is to provide a manual of basic microbiological techniques with applications for undergraduate students in diverse areas, including the biological sciences, the allied health sciences, agriculture, environmental science, nutrition, pharmacy, and various preprofessional programs. This manual contains 57 thoroughly class-tested exercises covering every area of microbiology. Most exercises require about 1½ hours of laboratory time. By selecting an appropriate combination of exercises, the instructor can provide learning experiences to meet the needs of a particular course.

Our Approach

Our goal in writing this manual has been twofold—to teach microbiological techniques and to show students the importance of microbes in our daily lives and their central roles in nature. *All* of the laboratory techniques, thinking skills, and safety procedures recommended by the American Society for Microbiology are included in this manual.

Laboratory safety is our primary concern. Many students are preparing for work in a clinical environment and need to learn to handle biologically contaminated materials. Students preparing for work in biotechnology and other laboratories must master aseptic techniques to avoid contamination of their samples. Students must not only learn but also practice these safety techniques so that safety becomes part of their professional behavior. We have followed the Centers for Disease Control and Prevention guidelines for the safe handling of microbes and human body fluids. Students are instructed to work only with their own body fluids. We have included a safety contract that students can hand in to their instructors to indicate that they understand safety requirements. See the section entitled Specific Hazards on page xii and the following sections of the Introduction for specific safety suggestions. At key points in the exercises, safety boxes appear. These are marked with either a biohazard logo ☣ or a general safety logo ⚠ indicating appropriate safety techniques.

Almost every exercise includes an actual experiment requiring students to analyze data. We hope in this way to promote *analytical reasoning* and to make laboratory sessions interesting for students as well as to provide a variety of opportunities for *reinforcement* of the technical skills they have learned. Each exercise has a series of Critical Thinking questions to enhance students' investigative skills. To further demonstrate some practical uses of microbiology, we have frequently included material with direct application to procedures performed in clinical and commercial laboratories.

Scope and Sequence

This manual is divided into 13 parts. The introduction to each part explains the unifying theme for that part. Exercises in the first three parts provide sequential development of fundamental techniques. The remaining exercises are as independent as possible to allow the instructor to select the most desirable sequence. A Techniques Required section preceding each experiment lists prerequisites from earlier exercises.

Part One, Microscopy, emphasizes observation through the microscope. Practice in use and care of the microscope is followed by observations of microbes, to familiarize students with their sizes. Phase-contrast and brightfield microscopy are used to examine living material.

Part Two, Staining Methods, begins with an explanation of how to handle bacterial cultures and teaches the use of the most common stains, including preparation of stained samples and their examination. A highly reliable capsule stain is included. Exercise 8, Morphologic Unknown, introduces the concept of unknowns and can be used to test knowledge of staining methods.

Part Three, Cultivation of Bacteria, stresses aseptic technique and covers the isolation of bacterial strains and the maintenance of bacterial cultures. An exercise dealing with special media prepares the student for the next part.

Part Four, Microbial Metabolism, includes five exercises on bacterial metabolism that provide the tools for Exercises 18, 33, and 51, consisting of unknown identifications. These exercises are especially useful for learning principles of metabolism. Exercise 18, Rapid Identification Methods, demonstrates numerical identification.

Part Five, Microbial Growth, deals with the effects of environmental conditions, such as temperature and the presence of oxygen, on growth. Biofilms are grown and observed in Exercise 21.

Part Six, Control of Microbial Growth, provides practical applications of concepts of microbial growth. Methods of controlling unwanted microbes in food or in a clinical environment are examined in this part.

Part Seven, Microbial Genetics, is of particular interest because of recent notable advances in this field. Exercise 27 is a unique experiment in which students look at expression in two operons using green fluorescent protein. Exercise 28 demonstrates the isolation of bacterial mutants. Exercise 29 allows students to isolate DNA and transform cells without special equipment. DNA fingerprinting is introduced in Exercise 30. Transformation by an antibiotic resistance plasmid is performed in Exercise 31. Restriction enzyme digestion and agarose gel electrophoresis are used to analyze the plasmid. In Exercise 32, suspected chemical carcinogens are tested by the Ames test.

Part Eight, The Microbial World, examines the diversity of microorganisms. Information and techniques learned in previous exercises are used to identify a bacterial unknown in Exercise 33. Cyanobacteria are included in Exercise 36, Phototrophs: Algae and Cyanobacteria. The morphology and ecological niches of free-living eukaryotic organisms studied in microbiology are examined in Exercises 34 through 37.

Part Nine, Viruses, provides an opportunity to isolate, cultivate, and quantify bacteriophages. Students determine the host range of a plant virus in Exercise 39.

Part Ten, Interaction of Microbe and Host, introduces basic concepts of epidemiology. Methods of tracking and identifying causes of infectious diseases are practiced in the exercises in this part.

Part Eleven, Immunology, covers the host's response to infectious disease with exercises on innate immunity and adaptive immunity (agglutination tests and the ELISA technique).

Part Twelve, Microorganisms and Disease, emphasizes procedures employed in the clinical laboratory. In Exercise 51, students identify an unknown from a simulated clinical sample.

Part Thirteen, Microbiology and the Environment, includes standard methods for the examination of food. The MUG test is used to examine water for bacteriological quality. Exercise 55, Microbes Used in the Production of Foods, offers an opportunity to study food microbiology. Exercise 56, Microbes in Soil: The Nitrogen and Sulfur Cycles, provides an example of biogeochemical cycles and soil microbiology. Exercise 57, Microbes in Soil: Bioremediation, is a unique exercise illustrating the use of bacteria for cleaning up environmental pollution.

The appendices at the end of the manual provide a convenient reference to techniques required in several exercises. Appendix H includes six identification keys to bacteria.

Organization of Each Exercise

The exercises in this manual may involve mastering a skill or procedure or understanding a particular concept. Most of the exercises are investigative by design, and the student is asked to analyze the experimental results and draw conclusions. The exercises are organized as follows:

- Each exercise begins with a section called *Objectives*, which lists skills or concepts to be mastered in that exercise. The objectives can be used to test mastery of the new material after completing the exercise.
- The *Background* provides definitions and explanations for each exercise. The student is expected to refer to a textbook for more detailed explanations of the concepts introduced in the laboratory exercises.
- *Materials* lists include supplies, media, and equipment needed for the exercise.
- *Cultures* lists identify the living organisms required for the exercise.
- *Techniques Required* gives a list of techniques needed to complete each exercise.
- *Procedure* then provides step-by-step instructions, stated as simply as possible and frequently supplemented with diagrams. Questions are occasionally asked in the Procedure section to remind the student of the rationale for a step.
- The *Laboratory Report* is designed to help students learn to collect and present data in a systematic fashion. Students are asked to write the *Purpose* of the exercise so they can relate the laboratory activity to their learning. Where appropriate, exercises ask students to formulate their *Hypotheses*. Tables are provided to record *Data* or *Results*. Occasionally, students are asked to write their *Expected Results* using information provided in the Background and their own experience. Students may be asked to write their *Conclusions* following the Data or Results. Usually, *Questions* in the Laboratory Report ask for interpretation of results. The Questions are designed to lead the student from a collection of data or observations to a conclusion. In most instances, the results for each student team will be unique; they can be compared with the information given in the Background and other references but will not be identical to those references. The range of questions in each exercise requires students to think about their results, recall facts, and then use this information to answer the questions. *Critical Thinking* questions are designed to help students use their new knowledge and practice analytic skills.

Illustrations

This book is generously illustrated with diagrams of procedures and 16 pages of photographs in full color. This section has been expanded and updated in this edition to include larger and more-detailed photos. There are two photo quizzes in the color section in which students identify an unknown.

For additional photographs of lab results, students can visit the laboratory supplement on the Microbiology Place web site (http://www.microbiologyplace.com). Photographs of media and techniques help students interpret their results without giving answers and actual lab results.

Preparation Guide

The comprehensive *Preparation Guide* (ISBN 0-8053-8293-3) provides the instructor with all the information needed to set up and teach a laboratory course with this manual. It includes the following:

- General instructions for setting up the lab.
- Information on obtaining and preparing cultures, media, and reagents.
- A master table showing the techniques required for each exercise.
- Cross-references for each exercise to specific pages in *Microbiology: An Introduction*, Ninth Edition.
- For each exercise: helpful suggestions, detailed lists of materials needed, and answers to all the questions in the student manual.

To make *Laboratory Experiments in Microbiology*, Eighth Edition, easy to use, we have carefully designed the experiments to use inexpensive, readily available, nonhazardous materials. Moreover, the exercises have been thoroughly tested in our classes in Minnesota and California by students with a wide variety of talents and interests. Our students have enjoyed their microbiology laboratory experiences; we hope yours will, too!

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Reviewers

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Tony Yates, Seminole State College

A Special Note to Students

This book is for you. The study of microbiology is dynamic because of the diversity of microbes and the variability inherent in every living organism. Outside of the laboratory—on a forest walk or tasting a fine cheese—we experience the activities of microbes. We want to share our excitement for studying these small organisms. Enjoy!

TED R. JOHNSON
CHRISTINE L. CASE