

# Preface

*Benson's Microbiological Applications* has been the "gold standard" of microbiology laboratory manuals for over 30 years. This manual has a number of attractive features that resulted in its adoption in universities, colleges, and community colleges for a wide variety of microbiology courses. These features include user-friendly diagrams that students can easily follow, clear instructions, and an excellent array of reliable exercises suitable for beginning or advanced microbiology courses.

In revising the lab manual for the thirteenth edition, we have tried to maintain the proven strengths of the manual and further enhance it. We have updated the introductory material of the fungi, protozoa, and algae to reflect changes in scientific information. Finally, the names of microorganisms used in the manual are consistent with those used by the American Type Culture Collection. This is important for those users who rely on the ATCC for a source of cultures.

## Guided Tour Through a Lab Exercise

### Learning Outcomes

Each exercise opens with Learning Outcomes, which list what a student should be able to do after completing the exercise.

### Learning Outcomes

After completing this exercise, you should be able to

1. Prepare a negative stain of bacterial cells using the slide-spreading or loop-spreading techniques.
2. Use the negative stain to visualize cells from your teeth and mouth.
3. Discern different morphological types of bacterial cells in a negative stain.

### Introduction

The introduction describes the subject of the exercise or the ideas that will be investigated. It includes all of the information needed to perform the laboratory exercise.

**Bacteriophages** are viruses that infect bacterial cells. They were first described by Twort and d'Herelle in 1915 when they both noted that bacterial cultures spontaneously cleared and the bacteria-free liquid that remained could cause new cultures of bacteria to also clear. Because it appeared that the cultures were being "eaten" by some unknown agent, d'Herelle coined the term *bacteriophage*, which means "bacterial eater." Like all viruses, bacteriophages, or phages, for short,

### First and Second Periods

In many cases, instructions are presented for two or more class periods so you can proceed through an exercise in an appropriate fashion.

#### First Period

(Inoculations and Incubation)

Since six microorganisms and three kinds of media are involved in this experiment, it will be necessary for economy of time and materials to have each student work with only three organisms. The materials list for this

#### Second Period

(Culture Evaluations and Spore Staining)

Remove the lid from the GasPak jar. If vacuum holds the inner lid firmly in place, break the vacuum by sliding the lid to the edge. When transporting the plates and tubes to your desk *take care not to agitate the FTM tubes*. The position of growth in the medium can be easily changed if handled carelessly.

### Materials Needed

This section lists the laboratory materials that are required to complete the exercise.

#### Materials

- microscope slides
- broth cultures of *Staphylococcus*, *Streptococcus*, and *Bacillus*
- Bunsen burner
- wire loop
- marking pen
- slide holder (clothespin)

### Procedures

The procedures and methods provide a set of detailed instructions for accomplishing the planned laboratory activities.

#### Scrub Procedure

The two members of the class who are chosen to perform the surgical scrub will set up their materials near a sink for convenience. As one student performs the scrub, the other will assist in reading the instructions and providing materials as needed. The basic steps,

# Illustrations

Illustrations provide visual instructions for performing steps in procedures or are used to identify parts of instruments or specimens.

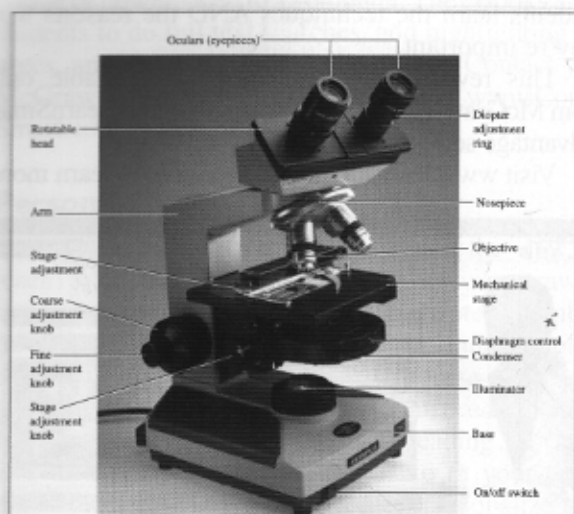


Figure 1.2 The compound microscope.  
© Charles D. Winters/Science Source.

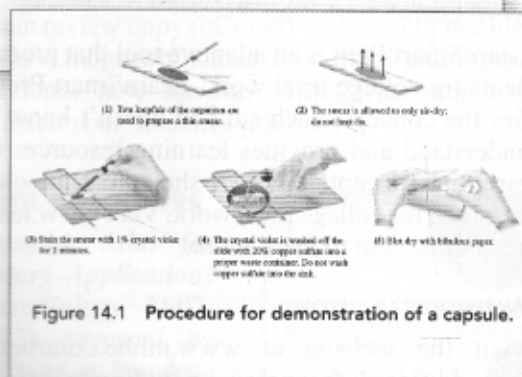


Figure 14.1 Procedure for demonstration of a capsule.

## Laboratory Reports

A Laboratory Report to be completed by students immediately follows most of the exercises. These Laboratory Reports are designed to guide and reinforce student learning and provide a convenient place for recording data. These reports include various types of review activities, tables for recoding observations and experimental results, and questions dealing with the analysis of such data.

As a result of these activities, students will increase their skills in gathering information by observation and experimentation. By completing all of the assessments in the Laboratory Reports, students will be able to determine if they accomplished all of the learning outcomes.

## Laboratory Report

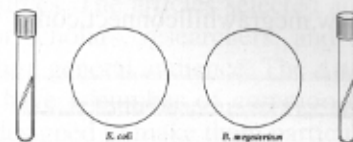
20

Student  
Date: / / Section:

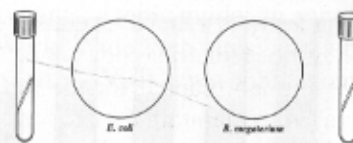
### 20 Preparation of Stock Cultures

#### A. Results

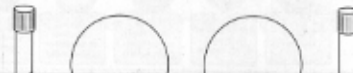
1. Appearance of slants after growth for 24 hours:



2. Appearance of culture after 4-6 weeks:



A. Room Temperature



## Laboratory Report

25

Student  
Date: / / Section:

### 25 Isolation of Phages from FIBs

#### A. Results

1. Plaque Size Factors

With a glass marking pencil, circle and label three plaques on one of the plain and record their sizes in millimeters at 1 hour intervals.

| TIME    | PLAQUE SIZE (millimeters) |              |              |
|---------|---------------------------|--------------|--------------|
|         | Plaque No. 1              | Plaque No. 2 | Plaque No. 3 |
| 1 hour  |                           |              |              |
| 2 hours |                           |              |              |
| 3 hours |                           |              |              |
| 4 hours |                           |              |              |
| 5 hours |                           |              |              |

- a. Were any plaques seen on the negative control plate?
- b. Do the plates show a progressive increase in number of plaques with increasing amount of *Hy-leuk* filtrate?
- c. Did the phage completely "help out" all bacterial growth on any of the plates? If so, which plate?

#### 2. Observations

Count all the plaques on each plate and record the counts in the following table. If the plaques are very numerous, use a colony counter and hand counting device. If this exercise is performed as a class project with individual students doing only one or two plates from a common *Hy-leuk* filtrate, record all counts on the table below as a table similar to the one below.

| Plate Number        | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|---------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>E. coli</i> (ml) | 0.0 | 0.4 | 0.7 | 1.0 | 1.3 | 1.6 | 1.9 | 2.2 | 2.5 | 2.8 |
| Plaque (ml)         | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1.0 |
| Number of plaques   |     |     |     |     |     |     |     |     |     |     |

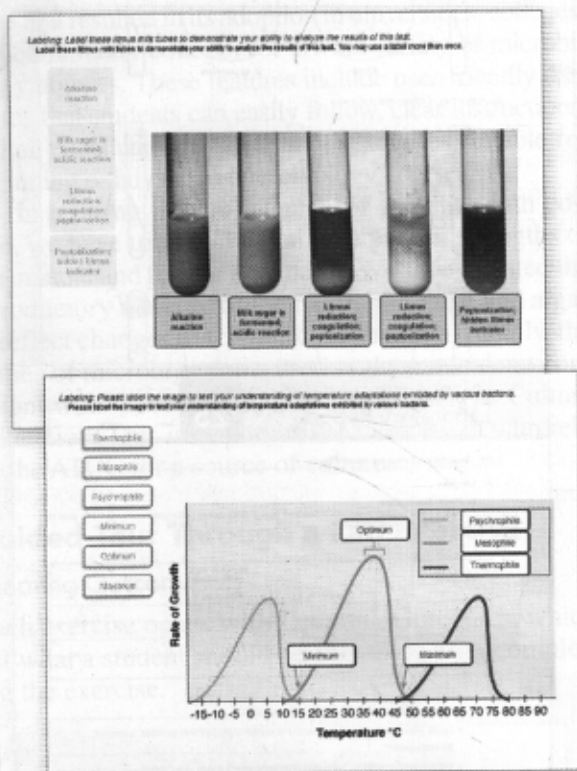
## Digital Tools for Your Lab Course

### McGraw-Hill Connect®

McGraw-Hill Connect allows instructors and students to use art and animations for assignments and lectures. A robust set of questions and activities are presented and aligned with the lab manual's exercises. As an instructor, you can edit existing questions and author entirely new problems. Track individual student performance—by question, assignment, or in relation to the class overall—with detailed grade reports. Instructors also have access to a variety of new resources, including assignable and gradable lab

## PREFACE

questions from the lab manual, additional pre- and post-lab activities, case study activities, interactive questions based on atlas images, lab skill videos, and more. In addition, digital images, PowerPoint slides, and instructor resources are available through Connect. Visit [www.mcgrawhillconnect.com](http://www.mcgrawhillconnect.com).



### Digital Lecture Capture

Tegrity Campus™ is a service that allows class time to be any time by automatically capturing every lecture in a searchable video format for students to review at their convenience. Educators know that the more students can see, hear, and experience class resources, the better they learn. Help turn all your students' study time into learning moments by supplying them with your lecture videos.

### New! LearnSmart Labs™

Fueled by LearnSmart—the most widely used and intelligent adaptive learning resource—LearnSmart Labs is a highly realistic and adaptive simulated lab experience that allows students to “do and think” like scientists.

LearnSmart Labs brings meaningful scientific exploration to students by giving them an adaptive environment in which to practice the scientific method, enabling them to make mistakes safely and to think critically about their findings. As a result, students come to lab better prepared and more likely to engage in the learning process.

The LearnSmart Labs questions, learning resources, and simulations were created with lab manual exercises in mind. This pairing gives you flexibility to implement technology in your lab course in a way that works for you. Whether you go all digital or try a hybrid approach, LearnSmart Labs will help your students learn the techniques AND the reasons why they're important.

This revolutionary technology is available only from McGraw-Hill Education as part of the LearnSmart Advantage series.

Visit [www.learnsmartadvantage.com](http://www.learnsmartadvantage.com) to learn more.



### New! LearnSmart Prep™

LearnSmart Prep is an adaptive tool that prepares students for college level work. LearnSmart Prep identifies the concepts each student doesn't know or fully understand and provides learning resources to teach essential concepts so he or she enters the classroom prepared for college level work. Visit [www.learnsmartadvantage.com](http://www.learnsmartadvantage.com) to learn more.

### Website

Visit the website at [www.mhhe.com/benson13e](http://www.mhhe.com/benson13e) for additional resources, including access to two additional exercises, Slime Mold Culture and White Blood Cell Study. For instructors, a password-protected instructor's manual and image library are available. The instructor's manual provides: (1) a list of equipment and supplies needed for performing all of the experiments, (2) procedures for setting up the experiments, and (3) answers to all the questions for the Laboratory Reports. The image library contains figures from the laboratory manual. Please contact your sales representative for additional information.

### Electronic Book—GO GREEN!

Green . . . it's on everybody's mind these days. It's not only about saving trees; it's also about saving money. If you or your students are ready for an alternative, McGraw-Hill eBooks offer a cheaper and eco-friendly alternative to traditionally printed textbooks and labo-



ratory manuals. This laboratory manual is available as an eBook at [www.CourseSmart.com](http://www.CourseSmart.com). At CourseSmart your students can take advantage of significant savings off the cost of a print textbook, reduce their impact on the environment, and gain access to powerful web tools for learning. CourseSmart eBooks can be viewed online or downloaded to a computer. The eBooks allow students to do full text searches, add highlighting and notes, and share notes with classmates. Contact your McGraw-Hill sales representative or visit [www.CourseSmart.com](http://www.CourseSmart.com) to learn more.

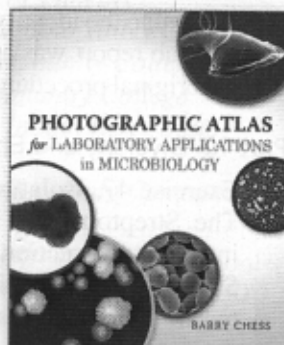
### Personalize Your Lab

Craft your teaching resources to match the way you teach! With McGraw-Hill Create™, [www.mcgrawhillcreate.com](http://www.mcgrawhillcreate.com), you can easily rearrange chapters, combine material from other content sources, and quickly upload content you have written like your course syllabus or teaching notes. Find the content you need in Create by searching through thousands of leading McGraw-Hill textbooks. Arrange your book to fit your teaching style. Create even allows you to personalize your book's appearance by selecting the cover and adding your name, school, and course information. Order a Create book and you'll receive a complimentary print review copy in 3–5 business days or a complimentary electronic review copy (eComp) via email in minutes. Go to [www.mcgrawhillcreate.com](http://www.mcgrawhillcreate.com) today and register to experience how McGraw-Hill Create empowers you to teach your students your way.

### Student Resources

#### *Photographic Atlas for Laboratory Applications in Microbiology* (0-07-737159-3), prepared by

Barry Chess at Pasadena City College, can be packaged with this laboratory manual. This beautifully prepared photo atlas contains more than 300 color



photos that bring the microbiology laboratory to life. The photo atlas is divided into eight major sections: staining techniques; cultural and biochemical tests; bacterial colonial morphology; bacterial microscopic morphology; fungi; protists; helminths; and hematology and serology. A picture is worth a thousand words, and this is definitely the case with this beautifully prepared atlas. Contact your McGraw-Hill sales representative for additional information and packaging options.

**Annual Editions: Microbiology 10/11** (0-07-738608-6) is a series of over 65 volumes, each designed to pro-

vide convenient, inexpensive access to a wide range of current articles from some of the most respected magazines, newspapers, and journals published today. **Annual Editions** are updated on a regular basis through a continuous monitoring of over 300 periodical sources. The articles selected are authored by prominent scholars, researchers, and commentators writing for a general audience. The **Annual Editions** volumes have a number of common organizational features designed to make them particularly useful in the classroom: a general introduction; an annotated table of contents; a topic guide; an annotated listing of selected World Wide Web sites; and a brief overview for each section. Visit [www.mhhe.com/clc](http://www.mhhe.com/clc) for more details.

### Changes to the Thirteenth Edition

- The thirteenth edition has a beautiful new design, with a striking color palette. Different-colored bars are used for Periods, Materials, Procedures, and Results.
- Each part opener now features a photograph depicting the theme of the exercises.
- For exercises that require several periods to complete, a clock icon has been included in the heading to indicate that results are required for the period and that further procedures may be necessary for the exercise.
- All cautions and warnings are denoted with a red bar to call attention to hazards associated with the exercise.
- A LearnSmart Lab icon has been included by the exercise title for those exercises where a LearnSmart Lab activity is available for all or part of that exercise.

#### Part 2, Survey of Microorganisms

- Exercise 6, Microbiology of Pond Water—Protists, Algae, and Cyanobacteria, has been revised to reflect changes in the taxonomy of these organisms. More emphasis is given to their role in disease and their importance in the environment and ecosystems.
- Exercise 7, Ubiquity of Bacteria, is now focused on the diversity and ubiquity of bacteria in the environment and how they are cultured. The morphology of bacterial cells has been moved to Exercise 12, Simple Staining.
- The Fungi, Exercise 8, has been revised to reflect changes in their taxonomy. Greater emphasis is given to their role in human disease, their importance as foods and in food production, and their role in environmental processes such as mineralization and the turnover of organic materials in ecosystems. The relationship between fungi that form a mycelium and yeasts is discussed.

#### Part 4, Staining and Observation of Microorganisms

- Exercises 11–18 on staining reactions have been reorganized and modified. Exercises have photographs in larger formats and some new photographs to show expected results. Also, each exercise now has its own set of results and questions, thus eliminating the necessity for students to record results and search for photos in later exercises.
- Exercise 15, Gram Staining, now includes new enlarged photomicrographs depicting gram-positive and gram-negative cells and align clearly with the procedure. More background on the history, importance, and theoretical basis of the Gram staining method has also been included. A corrected figure for the Gram stain steps is included as well. Procedures have slightly changed so that students have control bacteria on each slide to assist them in determining the success of their staining technique. The Laboratory Report has been enhanced, asking students to apply the concepts of cell shape and arrangement when evaluating their stained results.

#### Part 5, Culture Methods

- Enumeration of Bacteria: The Standard Plate Count, Exercise 21, now has photos of a set of serial dilution plates of a bacterial sample.

#### Part 6, Bacterial Viruses

- The steps in the infection of a bacterial cell by a bacteriophage have been revised in Exercise 23, Determination of a Bacteriophage Titer.
- Exercise 24, A One-Step Bacteriophage Growth Curve, includes major revisions that have been made to the background material and the procedure for enhanced student understanding and ease of implementation in the classroom. A figure of the one-step growth curve was added to the introductory material. Procedural figures were revised or deleted for clarity and alignment with changes to procedure. A graphing exercise and related analysis was added to the Laboratory Report.

#### Part 7, Environmental Influences and Control of Microbial Growth

- Exercise 27, Effects of Oxygen on Growth, has been revised to include the discussion of how oxygen influences growth and how it defines various classes of bacteria. Photographs showing the growth patterns of aerobes, anaerobes, microaerophiles, and facultative anaerobes in thioglycollate broth have replaced a diagram.

- A description of the organisms associated with human skin has been added to Exercise 33, Evaluation of Alcohol: Its Effectiveness as an Antiseptic.
- Antimicrobial Sensitivity Testing: The Kirby-Bauer Method, Exercise 34, has been updated with new information concerning health worker-acquired infections and the problem of antibiotic-resistant bacteria. New photographs of Kirby-Bauer plates showing sensitivity and resistance have been added, as well as photos showing how to measure the zone of inhibition.

#### Part 8, Identification of Unknown Bacteria

- Exercise 39, Physiological Characteristics: Oxidation and Fermentation Tests, now includes new photos of fermentation reactions (Durham tubes), the MRVP test, the citrate test, and the catalase.
- Added to Exercise 40, Physiological Characteristics: Hydrolytic and Degradative Reactions, are new photos of starch, casein, and fat hydrolysis.
- Physiological Characteristics: Multiple Test Media, Exercise 41, has new photos for SIM medium showing motility and hydrogen sulfide production. Enhanced photos of litmus milk reactions, including stormy fermentation, have also been added.
- The introductory material and separation outlines for Exercise 42, Use of *Bergey's Manual*, have been updated to reflect the current edition of *Bergey's* and the different volumes (both determinative and systematic). An additional challenge was added to this exercise aimed at teaching students how to use a table of test results and construct a flow chart to determine the identity of an unknown bacterium. A new lab report was added to this exercise for both the original procedure and the additional challenge.

#### Part 10, Diversity and Environmental Microbiology

- Exercise 47, Isolation of an Antibiotic Producer: The *Streptomyces*, includes the revision of the introductory section focusing on the role of the *Streptomyces* in antibiotic production. New photos of *Streptomyces* colonies on glycerol yeast extract agar have been added.

#### Part 13, Medical Microbiology

- Exercise 69, The Staphylococci: Isolation and Identification, includes new photos for Gram stain of staph, coagulase test, methyl green DNase test agar, and novobiocin sensitivity of *Staphylococcus epidermidis*.
- The Streptococci and Enterococci: Isolation and Identification, Exercise 70, includes a new photo of Gram stain of strep.



- Exercise 71, Gram-Negative Intestinal Pathogens, includes new photos of lactose fermenters on McConey and Eosin methylene blue agar.
- A Synthetic Epidemic, Exercise 72, has two new figures added to the introductory material, highlighting the two different categories of epidemics and the concept of herd immunity. Procedure B and its corresponding lab report section were revised to illustrate the concept of herd immunity.

#### Part 14, Immunology and Serology

- Exercise 74, Slide Agglutination Test for *S. aureus*, has a new photo of the C-reactive protein agglutination test showing positive and negative results.

### Acknowledgements

Our deepest gratitude to Judy Kaufman for her thorough review of the exercises and to Jill Kolodsick for her assistance in preparing the final draft of the manuscript and for her recommendations concerning the exercises.

We also wish to express our gratitude to Lisa Burgess for her excellent photographic contributions to the manual. The many new photos she provided will greatly improve the manual by providing clarity to many exercises.

The updates and improvements in this edition were guided by the helpful reviews and survey responses from the following instructors. Their input was critical to the decisions that shaped this edition.

Debra Albright *University of Phoenix*  
 Lorrie Burnham *San Bernardino Valley College*  
 Jessica Cofield *Craven Community College*  
 Iris Cook *Westchester Community College*  
 Adrienne Cottrell-Yongye *Indian River State College*  
 Amy Warena Czura *Suffolk County Community College Eastern Campus*  
 Angela Edwards *Trident Technical College*  
 Mary Farone *Middle Tennessee State University*  
 Deborah Fautot *University of Kansas*

Rebecca Giorno-McConnell *Louisiana Tech University*  
 Dale Harrington *Caldwell Community College and Technical Institute, Watauga Campus*  
 Betsy Hogan *Trident Technical College*  
 Jessica Jean *Capital Community College*  
 Mustapha Lahrach *Hillsborough Community College SouthShore Campus*  
 Jeffrey D. Leblond *Middle Tennessee State University*  
 Steven McConnell *Southeast Community College*  
 Thomas M. McNeilis *Dixie State College of Utah*  
 Caroline McNutt *Schoolcraft College*  
 Lathika Moragoda *Henry Ford Community College*  
 Christian Nwamba *Wayne County Community College District*  
 Teresa P. Palos *El Camino College*  
 Anita Denise Patterson *Northeast Alabama Community College*  
 Charles Pumpuni *Northern Virginia Community College*  
 Yilei Qian *Indiana University South Bend*  
 Pushpa Samkutty *Southern University*  
 Austin Smith *Jones County Junior College*  
 Davood Soleymani *California State University—Dominguez Hills*  
 Jamie Welling *South Suburban College*  
 Narinder Whitehead *Capital Community College*

We would like to thank all the people at McGraw-Hill for their tireless efforts and support with this project. They are professional and competent and always a pleasure to work with on this manual. A special and deep thanks to Darlene Schueller, our product developer. Once again, she kept the project focused, made sure we met deadlines and made suggestions that improved the manual in many ways. Thanks as well to Amy Reed, brand manager; Laura Bies, content project manager; Nichole Birkenholz, buyer; and Margarite Reynolds, designer, and many who worked "behind the scenes."