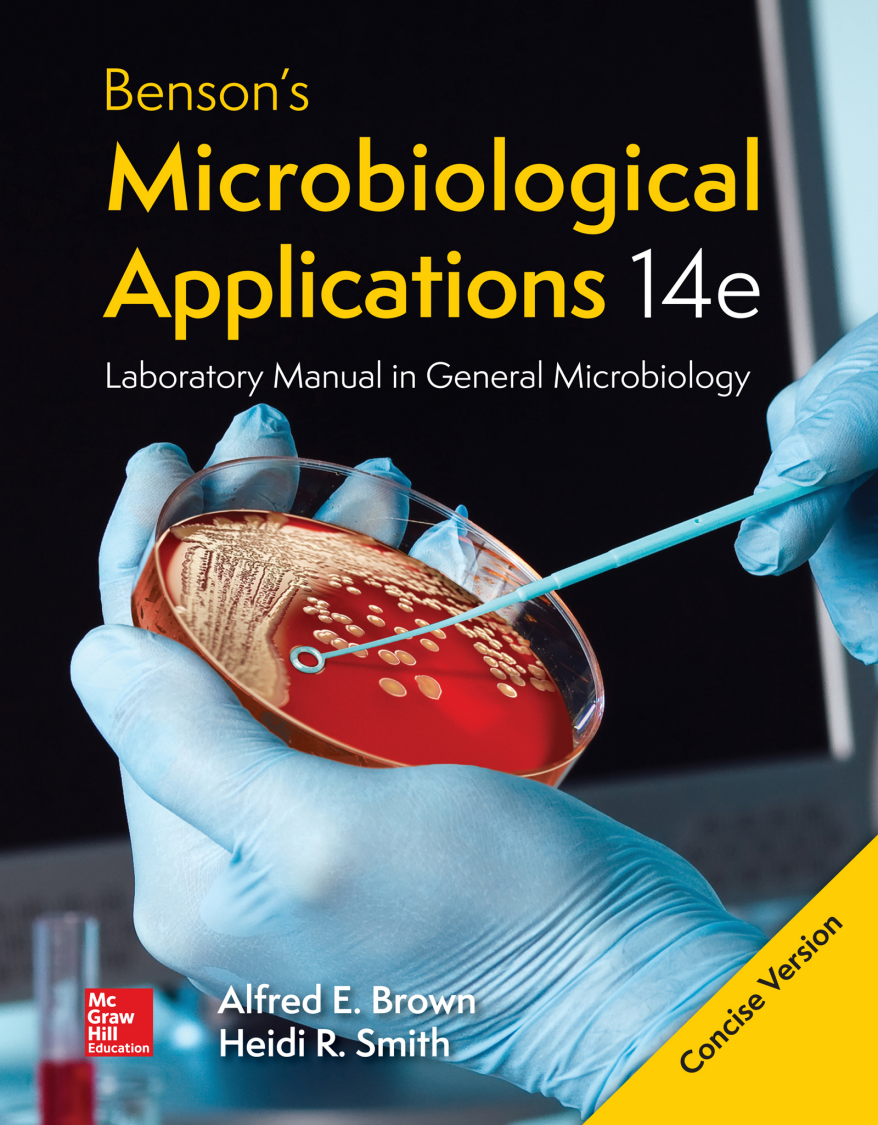
Benson's

Microbiological Applications 14e

Laboratory Manual in General Microbiology



Alfred E. Brown
Heidi R. Smith

Mc Graw Hill Education

Concise Version

CONCISE VERSION

Benson's Microbiological Applications

Laboratory Manual in General Microbiology

Fourteenth Edition

Alfred E. Brown
Emeritus Professor, Auburn University

Heidi R. Smith
Front Range Community College

Mc Graw Hill Education

BENSON'S MICROBIOLOGICAL APPLICATIONS: LABORATORY MANUAL IN GENERAL MICROBIOLOGY, CONCISE VERSION, FOURTEENTH EDITION

This book is printed on acid-free paper.

1 2 3 4 5 6 7 8 9 RMN 21 20 19 18 17 16

ISBN 978-1-259-70523-6
MHID 1-259-70523-4

Chief Product Officer, SVP Products & Markets: *G. Scott Virkler*
Vice President, General Manager, Products & Markets: *Marty Lange*
Managing Director: *Lynn Breithaupt*
Brand Manager: *Marija Magner*
Director, Product Development: *Rose Koos*
Product Developer: *Darlene M. Schueller*
Director of Digital Content: *Michael G. Koot, PhD*
Digital Product Analyst: *Jake Theobald*
Product Development Coordinator: *Andrea Eboh*
Marketing Manager: *Kristine Rellihan*
Director, Content Design & Delivery: *Linda Meehan-Avenarius*
Content Project Managers: *Mary Jane Lampe (Core) / Keri Johnson (Assessment)*
Content Licensing Specialists: *Melissa Homer (Image) / Lorraine Buczek (Text)*
Buyer: *Laura Fuller*
Designer: *Matt Backhaus*
Cover Image: © *Shutterstock/angellodeco; By R Parulan Jr./Getty Images*
Compositor: *MPS Limited, A Macmillan Company*
Typeface: *STIX MathJax Main 11/12*
Printer: *R. R. Donnelley*

www.mhhe.com

About the Authors

Alfred Brown

Emeritus Professor of Microbiology
Auburn University

B.S. Microbiology, California State College, Long Beach

Ph.D. Microbiology, UCLA

Teaching Dr. Brown has been a member of the American Society for Microbiology for 50 years, and he has taught various courses in microbiology over a teaching career that spans more than 30 years. Courses have included general microbiology, medical microbiology, microbial physiology, applied and environmental microbiology, photosynthesis, microbiological methods, and graduate courses such as biomembranes. In 2008, Dr. Brown retired from the Auburn University faculty as an emeritus professor of microbiology.

Administration During his tenure at Auburn University, Dr. Brown served as the director of the University Electron Microscope Facility. He also served as the chair of the Department of Botany and Microbiology and the chair of the Department of Biological Sciences.

Research My research has focused on the physiology of the purple nonsulfur bacteria. This has involved how bacteriochlorophyll and photosynthetic membrane synthesis are coordinated. Herbicides, such as atrazine, have been used to determine the binding site for ubiquinone in photosynthetic electron transport. Binding occurs on the L-subunit, a protein in the photosynthetic reaction center. Resistance to atrazine involves a single amino acid change in the L-subunit that prevents the herbicide from binding to the protein and inhibiting electron transport. This is comparable to how atrazine inhibits electron transport in plants and how resistance to these herbicides develops. My laboratory also investigated how the sulfonylurea herbicides inhibit acetolactate synthase, a crucial enzyme in the pathway for branched-chain amino acids. Recently, I consulted for a company that manufactures roofing shingles. Because of the presence of calcium carbonate in shingles, cyanobacteria can easily grow on their surface, causing problems of contamination. My laboratory isolated various species of cyanobacteria involved in the problem and taxonomically characterized them. We also tested growth inhibitors that might be used in their control.

Dr. Brown and his wife have traveled extensively in Europe since his retirement. His three children have followed him in science, having earned doctorates in physics, chemistry and anatomy.

Heidi Smith

Front Range Community College
B.A. Biology/Pre-Medicine, Taylor University, IN

M.S. Biology, Bowling Green State University, OH

Heidi Smith is the lead faculty for microbiology at Front Range Community College in Fort Collins, CO, and teaches a variety of biology courses each semester including microbiology, anatomy/physiology, and biotechnology. Heidi also serves as the principal investigator on a federal grant program designed to increase student success in transfer and completion of STEM degrees at the local university. In that role, Heidi works directly with students to train them for and support them through summer undergraduate research experiences.

Student success is a strategic priority at FRCC and a personal passion of Heidi's. She continually works to develop professionally in ways that help her do a better job of reaching this important goal. During the past 7 years, Heidi has had the opportunity to collaborate with faculty throughout the United States in developing digital tools, such as SmartBook, LearnSmart Labs, and Connect, that measure and improve student learning outcomes. This collaborative experience with these tools has revolutionized her approach to teaching in both face-to-face and hybrid courses. The use of digital technology has given Heidi the ability to teach courses driven by real-time student data and with a focus on active learning and critical thinking activities.

Heidi is an active member of the American Society for Microbiology. She has presented instructional technology and best online and face-to-face teaching practices on numerous occasions at the annual conference for undergraduate educators. She also served as a member of the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology Education, assisting in the identification of core microbiology concepts as a guide to undergraduate instruction.

Off campus, Heidi spends as much time as she can enjoying the beautiful Colorado outdoors with her husband and three young children.

Contents

Indicates a LearnSmart Lab® activity is available for all or part of this exercise. For more information, visit http://www.mhhe.com/LearnSmartLabsBiology/

Preface

Benson's Microbiological Applications has been the gold standard of microbiology laboratory manuals for over 35 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 fourteenth edition, we have tried to maintain the proven strengths of the manual and further enhance it. We have updated the introductory material in many exercises to reflect changes in scientific information and increase relevancy for students. Critical thinking questions have also been added to increase the Bloom's level of the laboratory reports. Finally, the names and biosafety levels 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 thin smear of bacterial cells and stain them with a simple stain.
2. Understand why staining is necessary to observe bacteria with a brightfield microscope.
3. Observe the different morphologies of bacterial cells.

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. The fourteenth edition has improved its student relevancy message within these introductions, explaining to students why they should care about the lab.

The recombinant plasmid pGLO used in this exercise contains a gene of interest known as the GFP gene (figure 50.1). This gene was isolated from a marine jellyfish and introduced into a bacterial plasmid using recombinant DNA techniques. The GFP gene codes for a *green fluorescent protein* that is visible when expressed by bacterial colonies that possess the plasmid.

In this exercise, the presence of the GFP gene on the pGLO plasmid will be verified by two molecular

Acquisition of genetic material, in addition to mutation of genes and loss of unnecessary genes, is an important part of bacterial adaptation and evolution. In many cases, acquisition of new genes (i.e., antibiotic resistance) may mean the difference between survival and death of an organism in the environment. Thus, bacteria have developed various mechanisms, includ-

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 period indicates how the organisms will

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

per student:

- 1 tube of nutrient broth
- 1 petri plate of trypticase soy agar (TSA)
- 1 sterile cotton swab
- Sharpie marking pen

per two or more students:

- 1 petri plate of blood agar

Procedures

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

Procedures

If your microscope has three objectives, you have three magnification options: (1) low-power, or 100× total magnification, (2) high-dry magnification, which is 400× total with a 40× objective, and (3) 1000× total magnification with a 100× oil immersion objective.

Whether you use the low-power objective or the oil immersion objective will depend on how much

Illustrations

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

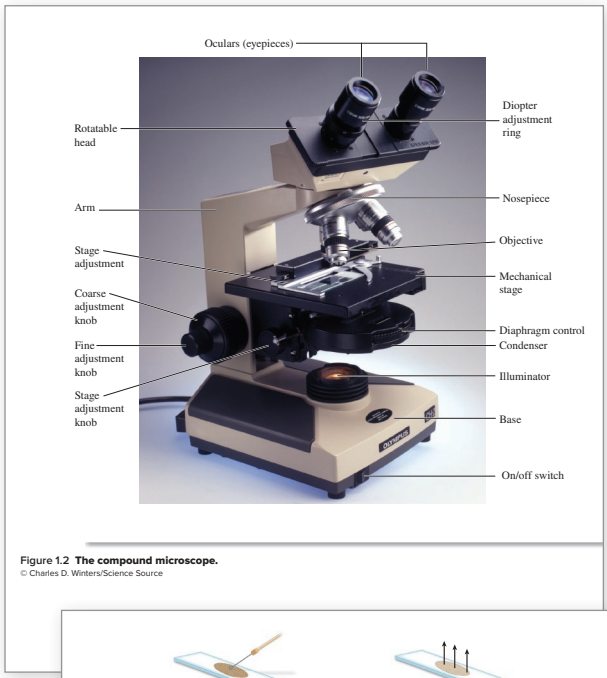


Figure 1.2 **The compound microscope.**
© Charles D. Winters/Science Source

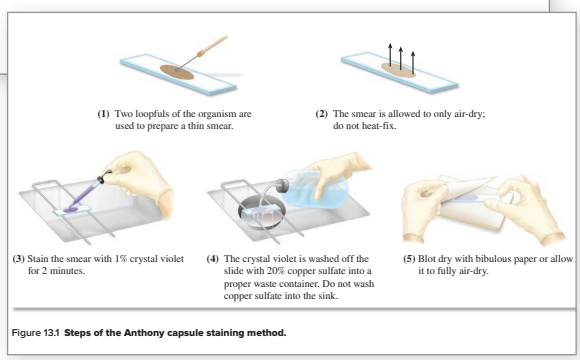
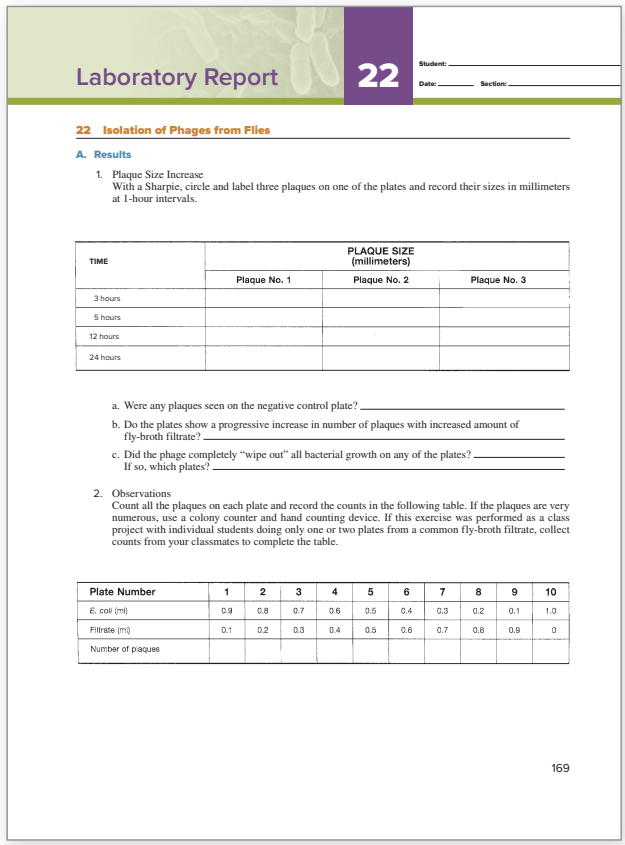


Figure 13.1 **Steps of the Anthony capsule staining method.**

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 recording 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 22

Student: _____
Date: _____ Section: _____

22 Isolation of Phages from Flies

A. Results

1. Plaque Size Increase
With a Sharpie, circle and label three plaques on one of the plates and record their sizes in millimeters at 1-hour intervals.

TIME	PLAQUE SIZE (millimeters)		
	Plaque No. 1	Plaque No. 2	Plaque No. 3
3 hours			
5 hours			
12 hours			
24 hours			

a. Were any plaques seen on the negative control plate? _____
b. Do the plates show a progressive increase in number of plaques with increased amount of fly-broth filtrate? _____
c. Did the phage completely "wipe out" all bacterial growth on any of the plates? _____ If so, which plates? _____

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 was performed as a class project with individual students doing only one or two plates from a common fly-broth filtrate, collect counts from your classmates to complete the table.

Plate Number	1	2	3	4	5	6	7	8	9	10
E. coli (ml)	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	1.0
Filtrate (ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0
Number of plaques										

169

Safety

In the fourteenth edition, we have followed the recent recommendations of the American Society for Microbiology concerning the biosafety levels of organisms used in the exercises in this laboratory manual. The Basic Microbiology Safety section in the introductory pages of the lab manual has been completely revised to align with ASM's *Guidelines for Biosafety in Teaching Laboratories* released in 2012. The BSL classifications of all organisms used in each exercise have been updated according to ATCC. Where possible, BSL-1 organisms replace BSL-2 organisms in many exercises. However, in some exercises, it is necessary to use BSL-2 organisms such as specific staphylococci, streptococci, and some of the *Enterobacteriaceae* due to specific tests, stains, and concepts that involve these organisms. For these exercises, we recommend that safety procedures be followed as suggested by ASM, such as the use of biosafety cabinets.

Changes to This Edition

Exercises

- Many photographs and some diagrams have been replaced, upgraded, or revised for clarity.

- Even if the procedure itself did not change, the text was revised to clarify areas where students traditionally struggle through an exercise.
- New critical thinking questions have been added to the end of many of the exercises.

Part 2 Survey of Microorganisms

- Updated diagram of Domains Bacteria, Archaea, and Eukarya.

Exercise 5 Microbiology of Pond Water

- Revision of introductory material.

Part 3 Manipulation of Microorganisms

Exercise 9 Pure Culture Techniques

- A photo of a pour plate was added with a description of surface versus subsurface colony growth.

Part 4 Staining and Observation of Microorganisms

Exercise 13 Capsular Staining

- A new photo of a capsule stain replaces the old photos.
- The introductory material was revised to explain how each step of the staining process works to allow for visualization of the capsule.

Exercise 15 Spore Staining: Two Methods

- Added a new photo of a spore stain.
- The procedures for the various staining methods were rearranged and revised for greater clarity.

Part 7 Environmental Influences and Control of Microbial Growth

Exercise 24 Effects of Oxygen on Growth

- Revised line art and photos throughout the exercise to clarify oxygen classifications.

Exercise 25 Temperature: Effects on Growth

- Added a figure depicting microbial temperature classification groups.
- Updated temperature ranges to most currently accepted upper and lower limits.

Exercise 28 Ultraviolet Light: Lethal Effects

- Replaced line art figure of UV lamp with photo of plate half covered by white card.

Part 8 Identification of Unknown Bacteria

Exercise 34 Morphological Study of an Unknown Bacterium

- New, easy-to-use descriptive chart for unknown identification.
- Added new figure of possible endospore positions in bacterial cell.

Exercise 36 Physiological Characteristics: Oxidation and Fermentation Tests

- Rearranged procedural sections and the process figures to make it easier for students to complete all of the required inoculations for both controls and unknown test bacteria.
- Added a flowchart to explain the steps of the nitrate reduction test and the correct analysis after each step.

Exercise 37 Physiological Characteristics: Hydrolytic and Degradative Reactions

- Revised figures, photos, and their associated captions to facilitate better student analysis of results for each of these tests.

Exercise 38 Physiological Characteristics: Multiple Test Media

- Removed litmus milk test.

Part 9 Miniaturized Multitest Systems

Exercise 40 *Enterobacteriaceae* Identification: The API® 20E System

- Simplified the tabulation process and enlarged the photo for easier analysis and identification by students.

Exercise 41 *Enterobacteriaceae* Identification: The EnteroPluri-*Test* System

- Completely rewrote this exercise to align with newly available test system from Becton-Dickinson due to discontinuance of the Entero-tube II system.

Part 10 Applied Microbiology

Exercise 44 Bacteriological Examination of Water: Most Probable Number Determination

- Updated introduction to the exercise.
- Revised the MPN table to make it easier for students to analyze and understand their results.

Exercise 46 Reductase Test

- The introductory section to the exercise has been revised and updated.

Exercise 49 Microbiology of Alcohol Fermentation

- The introduction to the exercise has been revised, emphasizing the history of fermentation and how fermentation is a means to preserve foods.
- Added an explanation of how off-flavors produced by sulfides occur in the fermentation of

wines and how they can be monitored by a lead acetate strip placed in the fermentation flask.

Part 11 Bacterial Genetics and Biotechnology

- This section was added to provide two exercises that are appropriate for general microbiology students. These exercises can be done individually or the techniques of PCR, gel electrophoresis, and bacterial transformation can be done as a connected sequence.

Part 12 Medical Microbiology

Exercise 52 The Staphylococci: Isolation and Identification

- Upgraded photos of mannitol salt agar.

Exercise 53 The Streptococci and Enterococci: Isolation and Identification

- Upgraded photos of blood agar plate hemolysis.

Part 13 Immunology and Serology

Exercise 59 Enzyme-Linked Immunosorbent Assay (ELISA)

- Added this exercise which simulates the commonly used screening test for HIV.

Exercise 60 Blood Grouping

- Added table figure to clearly explain the ABO groups.
- Revised introductory material, adding information on the Rh factor and the use of RhoGAM in Rh-negative mothers to prevent hemolytic disease in newborns.
- Revised the procedure for the use of artificial blood available in kits that can be purchased from biological supply companies. The use of finger sticks by students to collect blood and the use of human blood in laboratories has become a serious safety issue.

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 Marija Magner, brand manager; Mary Jane Lampe, content project manager; Kristine Rellihan, marketing manager; Matt Backhaus, designer; Keri Johnson, assessment content project manager; and Melissa Homer and Lorraine Buczek, content licensing specialists; and many who worked "behind the scenes."

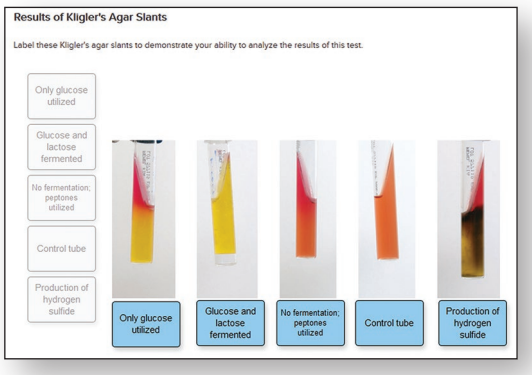
Digital Tools for Your Success

Save Time with Auto-Graded Assessments. Gather Powerful Performance Data.

McGraw-Hill Connect for Benson's Microbiological Applications Laboratory Manual in General Microbiology provides assignment and assessment solutions, connecting your students with the tools and resources they'll need to achieve success.

Homework and Assessment

With **Connect for Benson's Microbiological Applications Laboratory Manual in General Microbiology,** you can deliver auto-graded assignments, quizzes, and tests online. Choose from a robust set of interactive questions and activities using high-quality art from the lab manual and animations. Assignable content is available for every Learning Outcome and is categorized according to the ASM Objectives. As an instructor, you can edit existing questions and author entirely new ones.



© McGraw-Hill Education/Auburn University Photographic Services

Instructor Resources

Customize your lecture with tools such as animations and images. An instructor's manual is also available.

Lecture Capture

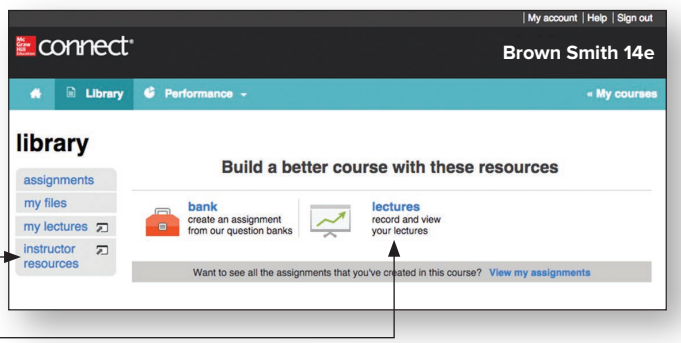
McGraw-Hill Tegrity® records and distributes your class lecture with just a click of a button. Students can view this lecture material anytime, anywhere via computer or mobile device. The material is indexed as you record, so students can use keywords to find exactly what they want to study.

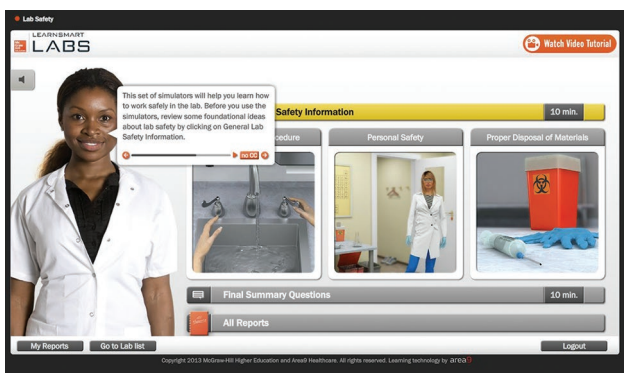
LearnSmart Labs® is an optional adaptive simulated lab experience that brings meaningful scientific exploration to students. Through a series of adaptive questions, LearnSmart Labs identifies a student's knowledge gaps and provides resources to quickly and efficiently close those gaps. Once students have mastered the necessary basic skills and concepts, they engage in a highly realistic simulated lab experience that allows for mistakes and the execution of the scientific method. LearnSmart Labs are currently being used as a pre-lab preparation assignment or as full lab replacement.

LearnSmart® Prep is an adaptive learning tool that prepares students for college-level work in Microbiology, and is included with Connect for Benson's Microbiological Applications purchases. LearnSmart Prep individually identifies concepts the student does not fully understand and provides learning resources to teach essential concepts so he or she enters the classroom prepared. Data-driven reports highlight areas where students are struggling.

Detailed Reports

Track individual student performance—by question, by assignment, or in relation to the class overall—with detailed grade reports. Integrate grade reports easily with your Learning Management Systems (LMS).





Learn more at connect.mheducation.com.

Basic Microbiology Laboratory Safety

Every student and instructor must focus on the need for safety in the microbiology laboratory. While the lab is a fascinating and exciting learning environment, there are hazards that must be acknowledged and rules that must be followed to prevent accidents and contamination with microbes. The following guidelines will provide every member of the laboratory the information required to assure a safe learning environment.

Microbiological laboratories are special, often unique environments that may pose identifiable infectious disease risks to persons who work in or near them. Infections have been contacted in the laboratory throughout the history of microbiology. Early reports described laboratory-associated cases of typhoid, cholera, brucellosis, and tetanus, to name a few. Recent reports have documented laboratory-acquired cases in laboratory workers and healthcare personnel involving *Bacillus anthracis, Bordetella pertussis, Brucella, Burkholderia pseudomallei, Campylobacter, Chlamydia,* and toxins from *Clostridium tetani, Clostridium botulinum,* and *Corynebacterium diphtheriae.* In 2011, the CDC traced an outbreak of *Salmonella* to several undergraduate microbiology laboratories, prompting further discussion about safety guidelines for the lab classroom setting.

The "Biohazard" symbol must be affixed to any container or equipment used to store or transport potentially infectious materials.

Courtesy of the Centers for Disease Control.

The term "containment" is used to describe the safe methods and procedures for handling and managing microorganisms in the laboratory. An important laboratory procedure practiced by all microbiologists that will guarantee containment is **aseptic technique,** which prevents workers from contaminating themselves with microorganisms, ensures that others and the work area do not become contaminated, and also ensures that microbial cultures do not become unnecessarily contaminated with unwanted organisms. Containment involves personnel and the immediate laboratory environment. Containment also guarantees that infectious agents do not escape from the laboratory and contaminate the environment external to the lab. Containment, therefore, relies on good microbiological technique and laboratory protocol as well as the use of appropriate safety equipment.

Biosafety Levels (BSL)

The biosafety level classifications of microorganisms represent the potential of the organism to cause disease and the conditions under which the organism should be safely handled. The Centers for Disease Control classifies organisms into four levels, which take into account many factors such as virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, and other factors. The four biosafety levels are described in the table on page xii.

All microorganisms used in the exercises in this manual are classified as BSL-1 or BSL-2. Note: Although some of the organisms that students will culture and work with are classified as BSL-2, these organisms may be laboratory strains that do not pose the same threat of infection as primary isolates of the same organism taken from patients in clinical samples. Hence, these laboratory strains can, in most cases, be handled using normal procedures and equipment found in the vast majority of student teaching laboratories. However, it should be emphasized that many bacteria are opportunistic pathogens, and therefore all microorganisms should be handled by observing proper techniques and precautions.

Each of the biosafety levels indicates that certain laboratory practices and techniques, safety equipment, and laboratory facilities should be used when working with organisms in that classification. Each combination is specifically appropriate for the operations performed and the documented or suspected routes of transmission of the infectious agents. In response to the *Salmonella* outbreaks in undergraduate laboratories, the American Society for Microbiology set out to define a clear set of safety practices for laboratories based on the use of BSL-1 or BSL-2 organisms. In 2013, the *Guidelines for Biosafety in Teaching Laboratories* was published in the *Journal of Microbiology Education*.

Standard Laboratory Practices (Based on ASM *Guidelines for Biosafety in Teaching Laboratories*)

BSL-1 Guidelines

Although BSL-1 organisms pose very little risk of disease for healthy students, they are still capable of causing infection under certain circumstances. These guidelines indicate the recommended best practices in the laboratory for the protection of students and the community.

Personal Protection

- It is recommended that lab coats be worn in the laboratory at all times. Lab coats can protect a student from contamination by microorganisms that he or she is working with and prevent

Biosafety Levels for Selected Infectious Agents

BIOSAFETY LEVEL (BSL)	TYPICAL RISK	ORGANISM
BSL-1	Not likely to pose a disease risk to healthy adults.	*Achromobacter denitrificans* *Alcaligenes faecalis* *Bacillus cereus* *Bacillus subtilis* *Corynebacterium pseudodiphtheriticum* *Micrococcus luteus* *Neisseria sicca* *Staphylococcus epidermidis* *Staphylococcus saprophyticus*
BSL-2	Poses a moderate risk to healthy adults; unlikely to spread throughout community; effective treatment readily available.	*Enterococcus faecalis* *Klebsiella pneumoniae* *Mycobacterium phlei* *Salmonella enterica var. Typhimurium* *Shigella flexneri* *Staphylococcus aureus* *Streptococcus pneumoniae* *Streptococcus pyogenes*
BSL-3	Can cause disease in healthy adults; may spread to community; effective treatment readily available.	*Blastomyces dermatitidis* *Chlamydia trachomatis* *Coccidioides immitis* *Coxiella burnetii* *Francisella tularensis* *Histoplasma capsulatum* *Mycobacterium bovis* *Mycobacterium tuberculosis* *Pseudomonas mallei* *Rickettsia canadensis* *Rickettsia prowazekii* *Yersinia pestis*
BSL-4	Can cause disease in healthy adults; poses a lethal risk and does not respond to vaccines or antimicrobial therapy.	Filovirus *Herpesvirus simiae* Lassa virus Marburg virus Ebola virus

contamination from stains and chemicals. At the end of the laboratory session, lab coats are usually stored in the lab in a manner prescribed by the instructor.

- You may be required to wear gloves while performing the lab exercises. They protect the hands against contamination by microorganisms and prevent the hands from coming in direct contact with stains and other reagents. This is especially important if you have open wounds.
- Wash your hands with soap and water before and after handling microorganisms.
- Safety goggles or glasses should be worn while you are performing experiments with liquid cultures, splash hazards, or while spread plating. They must also be worn when working with ultraviolet light to prevent eye damage because they block out UV rays.
- Sandals or open-toe shoes are not to be worn in the laboratory. Accidental dropping of objects or cultures could result in serious injury or infection.
- Lab coats, gloves, and safety equipment should not be worn outside of the laboratory unless properly decontaminated first.
- Students with long hair should tie the hair back to avoid accidents when working with Bunsen burners/open flames. Long hair can also be a source of contamination when working with cultures.
- Avoid wearing dangling jewelry or scarves to lab.
- If you are immune-compromised (including pregnancy) or provide care for someone who is immune-compromised, please consult with your physician about your participation in these laboratory exercises.

Laboratory Environment and Equipment

- Most importantly, read the exercise and understand the laboratory protocol before coming to laboratory. This way, you will be familiar with potential hazards in the exercise. Unless directed to do so, do not subculture any unknown organisms

isolated from the environment as they could be potential pathogens.
- Students should store all books and materials not used in the laboratory in areas or receptacles designated for that purpose. Only necessary materials such as a lab notebook and the laboratory manual should be brought to the student work area. Use only institution-provided writing instruments.
- Avoid handling personal items such as cell phones and calculators while performing laboratory exercises. Students must also avoid handling contact lenses or applying makeup while in the laboratory.
- Eating, drinking (including water), gum, chewing tobacco, and smoking are not allowed in the laboratory.
- Know the location of exits and safety equipment such as the eye wash and shower stations, first aid kit, and fire extinguisher in the event of an accident that requires the use of this equipment.
- The door to the laboratory must remain closed, and only enrolled students should be allowed to enter the laboratory classroom.
- Before beginning the activities for the day, work areas should be wiped down with the disinfectant that is provided for that purpose. Likewise, when work is finished for the day, the work area should be treated with disinfectant to ensure that any contamination from the exercise performed is destroyed. To avoid contaminating the work surface, do not place contaminated pipettes, loops/needles, or swabs on the work surface.
- Always use correct labeling procedures on all containers.
- If possible, use a microincinerator or disposable loops for transferring microorganisms from one container to another. If these are unavailable, please use extreme caution when working with the open flame of a Bunsen burner. The flame is often difficult to see.
- Caution is imperative when working with alcohol and open flames. Alcohol is highly flammable, and fires can easily result when using glass rods that have been dipped in alcohol.
- Always make sure the gas is turned off before leaving the laboratory.
- Pipetting by mouth is prohibited in the lab. All pipetting must be performed with pipette aids.
- Use test tube racks when transporting cultures throughout the laboratory.
- You may be required to sign a safety agreement stating that you have been informed about safety issues and precautions and the hazardous nature of microorganisms that you may handle during the laboratory course.

BSL-2 Guidelines

BSL-2 organisms pose a moderate risk of infections, but the diseases caused by these organisms are treatable and usually not serious. Before working with these organisms, students should already show proficiency in following all of the guidelines for BSL-1 organisms. Additional precautions that should be taken when working with BSL-2 organisms include:

- Wear face shields or masks, along with proper eye wear, when working with procedures that involve a potential splash hazard. Alternatively, conduct all work with these organisms in a biological safety cabinet.
- Laboratory coats are required when working with these organisms.
- Use microincinerators or disposable loops rather than Bunsen burners.

Emergencies

- Report all spills, accidents, or injuries immediately to the laboratory instructor.
- Do not handle broken glass with your hands.
- Follow institutional policy in documenting all injuries and other emergency situations.

Disposal of Laboratory Materials

Dispose of all contaminated materials properly and in the appropriate containers. Your instructor will give you specific instructions for your laboratory classroom.

- Biohazard containers—Biohazard containers are to be lined with clearly marked biohazard bags; disposable agar plates, used gloves, and any materials such as contaminated paper towels should be discarded in these containers; no glassware, test tubes, or sharp items are to be disposed of in biohazard containers.
- Sharps containers—Sharps, needles, and Pasteur pipettes should be discarded in these containers.
- Autoclave shelf, cart, or bin—Contaminated culture tubes and glassware used to store media and other glassware should be placed in these areas for decontamination and washing.
- Trash cans—Any noncontaminated materials, paper, or trash should be discarded in these containers. Under no circumstances should laboratory waste be disposed of in trash cans.
- Slides and broken glass may be disposed of in a sharps container, a beaker filled with disinfectant, or a labeled cardboard box. Listen carefully to your instructor's directions for these items.

Microorganisms Used or Isolated in the Lab Exercises in This Manual

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Alcaligenes faecalis ATCC 8750	Negative rod	Decomposing organic material, feces	1	26, 39
Bacillus coagulans ATCC 7050	Positive rod	Spoiled food, silage	1	48
Bacillus megaterium ATCC 14581	Positive rod	Soil, water	1	11, 12, 14, 15, 28, 47
Bacillus subtilis ATCC 23857	Positive rod	Soil, decomposing organic matter	1	24, 37
Candida glabrata ATCC 200918	Yeast	Human oral cavity	1	26
Chromobacterium violaceum ATCC 12472	Negative rod	Soil and water; opportunistic pathogen in humans	2	9
Citrobacter freundii ATCC 8090	Negative rod	Humans, animals, soil water; sewage opportunistic pathogen	1	54
Clostridium beijerinckii ATCC 25752	Positive rod	Soil	1	24
Clostridium sporogenes ATCC 3584	Positive rod	Soil, animal feces	1	24, 48
Corynebacterium xerosis ATCC 373	Positive rods, club-shaped	Conjunctiva, skin	1	11
Enterobacter aerogenes ATCC 13048	Negative rods	Feces of humans and animals	1	24, 36, 39, 44
Enterococcus faecalis ATCC 19433	Positive cocci in pairs, short chains	Water, sewage, soil, dairy products	2	24, 39, 53, 58
Enterococcus faecium ATCC 19434	Positive cocci in pairs, short chains	Feces of humans and animals	2	53, 58
Escherichia coli ATCC 11775	Negative rods	Sewage, intestinal tract of warm-blooded animals	1	8, 9, 14, 19, 21, 22, 24, 25, 26, 27, 29, 31, 36, 37, 38, 39, 44, 47, 48, 50, 51
Geobacillus stearothermophilus ATCC 12980	Gram-positive rods	Soil, spoiled food	1	25, 48
Halobacterium salinarium ATCC 33170	Gives gram-negative reaction; rods	Salted fish, hides, meats	1	27
Klebsiella pneumoniae ATCC 13883	Negative rods	Intestinal tract of humans; respiratory and intestinal pathogen in humans	2	13, 39
Streptococcus lactis ATCC 19435	Positive cocci in chains	Milk and milk products	1	11

Microorganisms Used or Isolated in the Lab Exercises in This Manual (continued)

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Micrococcus luteus ATCC 12698	Positive cocci that occur in pairs	Mammalian skin	1	9, 17, 29, 39
Moraxella catarrhalis ATCC 25238	Negative cocci that often occur in pairs with flattened sides	Pharynx of humans	1	14
Mycobacterium smegmatis ATCC 19420	Positive rods; may be Y-shaped or branched	Smegma of humans	1	16
Proteus vulgaris ATCC 29905	Negative rods	Intestines of humans, and animals; soil and polluted waters	2	17, 31, 37, 38, 39, 54
Pseudomonas aeruginosa ATCC 10145	Negative rods	Soil and water; opportunistic pathogen in humans	2	14, 31, 32, 36, 39
Saccharomyces cerevisiae ATCC 18824	Yeast	Fruit, used in beer, wine, and bread	1	26
Salmonella enterica subsp. enterica serovar *Typhimurium* ATCC 700720	Negative rods	Most frequent agent of *Salmonella* gastroenteritis in humans	2	39, 54, 56
Serratia marcescens ATCC 13880	Negative rods	Opportunistic pathogen in humans	1	9, 25, 39, 55
Shigella flexneri ATCC 29903	Negative rods	Pathogen of humans	2	54
Staphylococcus aureus ATCC 12600	Positive cocci, irregular clusters	Skin, nose, GI tract of humans, pathogen	2	11, 14, 23, 24, 29, 31, 32, 36, 37, 38, 47, 52, 53, 57
Staphylococcus epidermidis ATCC 14990	Positive cocci that occur in pairs and tetrads	Human skin, animals; opportunistic pathogen	1	12, 14, 16, 26, 27, 28, 39, 52
Staphylococcus saprophyticus ATCC 15305	Positive cocci that occur singly and in pairs	Human skin; opportunistic pathogen in the urinary tract	1	52
Streptococcus agalactiae ATCC 13813	Positive cocci; occurs in long chains	Upper respiratory and vaginal tract of humans, cattle; pathogen	2	53, 58
Streptococcus bovis ATCC 33317	Positive cocci; pairs and chains	Cattle, sheep, pigs; occasional pathogen in humans	1	53, 58
Streptococcus dysagalactiae subsp. equisimilis ATCC 12394	Positive cocci in chains	Mastitis in cattle	2	53

Microorganisms Used or Isolated in the Lab Exercises in This Manual (continued)

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Streptococcus mitis ATCC 49456	Positive cocci in pairs and chains	Oral cavity of humans	2	53
Streptococcus mutans ATCC 25175D-5	Positive cocci in pairs and chains	Tooth surface of humans, causes dental caries	1	53
Streptococcus pneumoniae ATCC 33400	Positive cocci in pairs	Human pathogen	2	53
Streptococcus pyogenes ATCC 12344	Positive cocci in chains	Human respiratory tract; pathogen	2	53, 58
Streptococcus salivarius ATCC 19258	Positive cocci in short and long chains	Tongue and saliva	1	53
Thermoanaerobacterium thermosaccharolyticum ATCC 7956	Negative rods; single cells or pairs	Soil, spoiled canned foods	1	48

PART

1

Microscopy

Although there are many kinds of microscopes available to the microbiologist today, only three types will be described here for our use: the brightfield, and phase-contrast microscopes. If you have had extensive exposure to microscopy in previous courses, this unit may not be of great value to you; however, if the study of microorganisms is a new field of study for you, there is a great deal of information that you need to acquire about the proper use of these instruments.

Microscopes in a college laboratory represent a considerable investment and require special care to prevent damage to the lenses and mechanical parts. A microscope may be used by several people during the day and moved from the work area to storage, which results in a much greater chance for damage to the instrument than if the microscope were used by only a single person.

The complexity of some of the more expensive microscopes also requires that certain adjustments be made periodically. Knowing how to make these adjustments to get the equipment to perform properly is very important. An attempt is made in the four exercises of this unit to provide the necessary assistance for getting the most out of the equipment.

Microscopy should be as fascinating to the beginner as it is to the professional of long standing; however, only with intelligent understanding can the beginner approach the achievement that occurs with years of experience.

This page intentionally left blank

Brightfield Microscopy

EXERCISE 1

Learning Outcomes

After completing this exercise, you should be able to

1. Identify the basic components of a brightfield microscope and understand the function of each component in proper specimen observation.
2. Examine a specimen using the low-power, high-dry, and oil immersion lenses.
3. Understand the proper use, care, and storage of a microscope.

A microscope that allows light rays to pass directly to the eye without being deflected by an intervening opaque plate in the condenser is called a ***brightfield microscope.*** This is the conventional type of instrument encountered by students in beginning courses in biology; it is also the first type to be used in this laboratory.

All brightfield microscopes have certain things in common, yet they differ somewhat in mechanical operation. Similarities and differences of various makes are discussed in this exercise so that you will know how to use the instrument that is available to you. Before attending the first laboratory session in which the microscope is used, read over this exercise and answer all the questions on the Laboratory Report. Your instructor may require that the Laboratory Report be handed in prior to doing any laboratory work.

Care of the Instrument

Microscopes represent considerable investment and can be damaged easily if certain precautions are not observed. The following suggestions cover most hazards.

Transport When carrying your microscope from one part of the room to another, use both hands to hold the instrument, as illustrated in figure 1.1. If it is carried with only one hand and allowed to dangle at your side, there is always the danger of collision with furniture or some other object. And, *under no circumstances should one attempt to carry two microscopes at one time.*

Figure 1.1 **The microscope should be held firmly with both hands while being carried.**

Clutter Keep your workstation uncluttered while doing microscopy. Keep unnecessary books and other materials away from your work area. A clear work area promotes efficiency and results in fewer accidents.

Electric Cord Microscopes have been known to tumble off of tabletops when students have entangled a foot in a dangling electric cord. Don't let the electric cord on your microscope dangle in such a way as to risk foot entanglement.

Lens Care At the beginning of each laboratory period, check the lenses to make sure they are clean. At the end of each lab session, be sure to wipe any immersion oil off the immersion lens if it has been used. More specifics about lens care are provided later in this exercise.

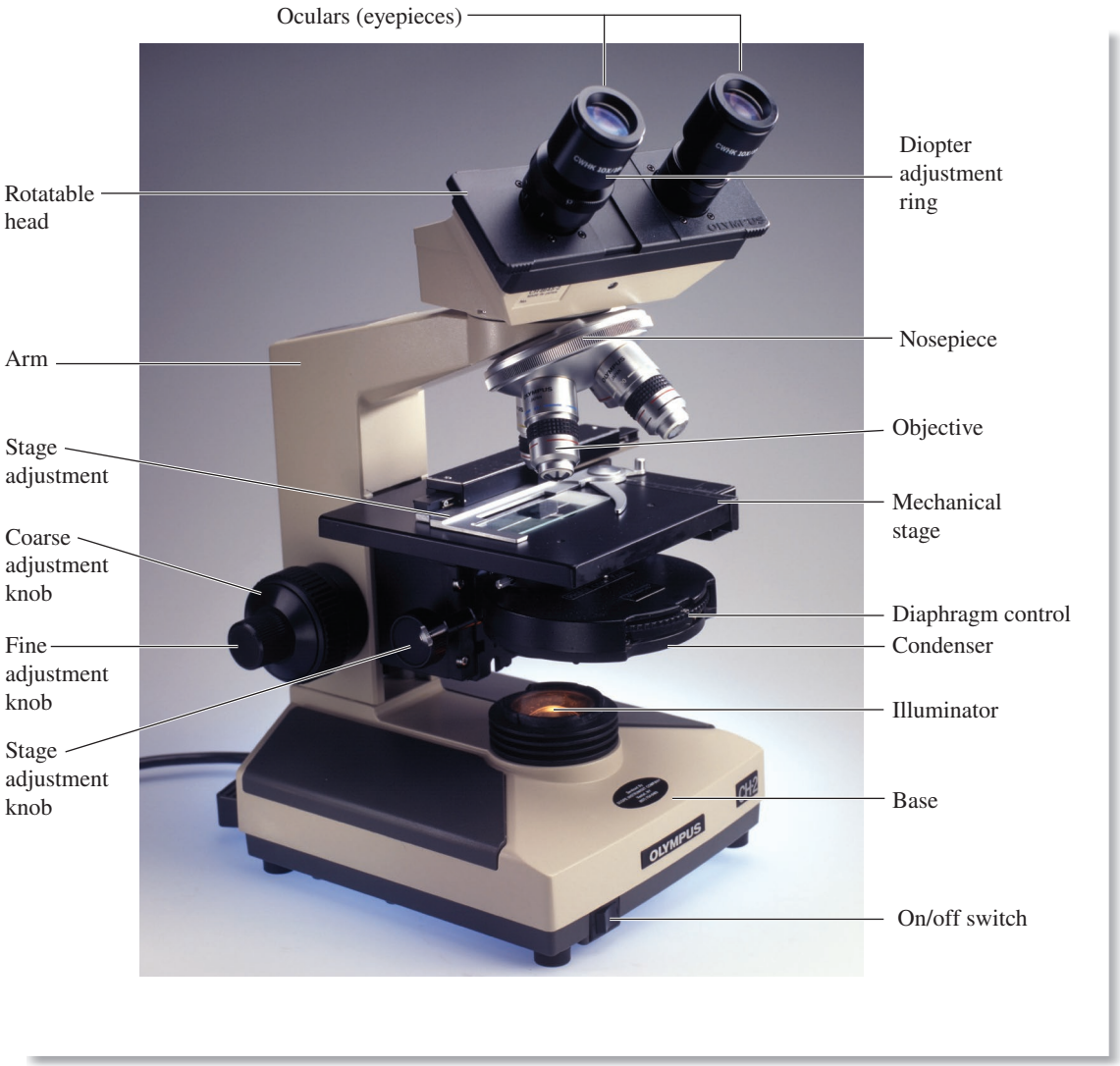


Figure 1.2 **The compound microscope.**

Dust Protection In most laboratories dustcovers are used to protect the instruments during storage. If one is available, place it over the microscope at the end of the period.

Components

Light Source In the base of most microscopes is positioned some kind of light source. Ideally, the lamp should have a **light intensity control** to vary the intensity of light. The microscope in figure 1.2 has a knurled wheel on the right side of its base to regulate the voltage supplied to the lightbulb.

Most microscopes have some provision for reducing light intensity with a **neutral density filter.** Such a filter is often needed to reduce the intensity of light below the lower limit allowed by the voltage control. On microscopes such as the Olympus CH2, one can simply place a neutral density filter over the light source in the base. On some microscopes a filter is built into the base.

Lens Systems All compound microscopes have three lens systems: the oculars, the objectives, and the condenser. Figure 1.3 illustrates the light path through these three systems.

The **ocular,** or eyepiece, is a complex piece, located at the top of the instrument, that consists of two or more internal lenses and usually has a magnification of 10×. Most modern microscopes (figure 1.2) have two ocular (binocular) lenses.

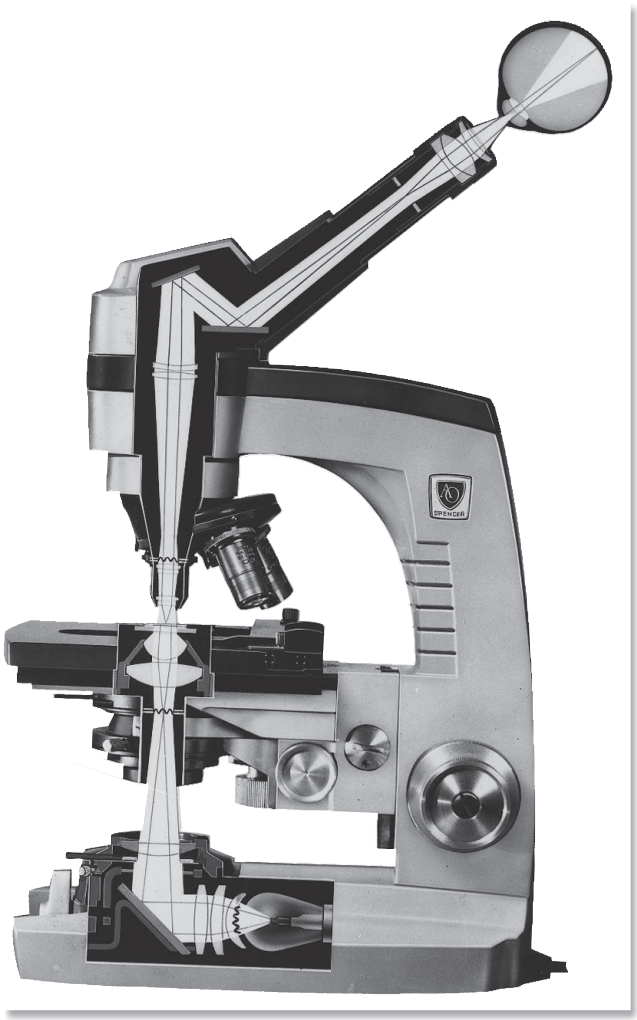


Figure 1.3 **The light pathway of a microscope.**

Three or more **objectives** are usually present. Note that they are attached to a rotatable **nosepiece,** which makes it possible to move them into position over a slide. Objectives on most laboratory microscopes have magnifications of 10×, 40×, and 100×, designated as **low-power, high-dry,** and **oil immersion,** respectively. Some microscopes will have a fourth objective for rapid scanning of microscopic fields that is only 4×.

The total magnification of a compound microscope is determined by multiplying the power of the ocular lens times the power of the objective lens used. Thus, the magnification of a microscope in which the oil immersion lens is being used is:

$$10 \times 100 = 1000$$

The object is now magnified 1000 times its actual size. The third lens system is the **condenser,** which is located under the stage. It collects and directs the light from the lamp to the slide being studied. Unlike the ocular and objective lenses, the condenser lens does not affect the magnifying power of the compound microscope. The condenser can be moved up and down by a knob under the stage. A **diaphragm** within the condenser regulates the amount of light that reaches the slide. Microscopes that lack a voltage control on the light source rely entirely on the diaphragm for controlling light intensity. On the Olympus CH2 microscope in figure 1.2, the diaphragm is controlled by turning a knurled ring. On some microscopes, a diaphragm lever is present. Figure 1.2 illustrates the location of the condenser and diaphragm.

Focusing Knobs The concentrically arranged **coarse adjustment** and **fine adjustment knobs** on the side of the microscope are used for bringing objects into focus when studying an object on a slide. On some microscopes, these knobs are not positioned concentrically as shown here.

Ocular Adjustments On binocular microscopes, one must be able to change the distance between the oculars and to make diopter changes for eye differences. On most microscopes, the interocular distance is changed by simply pulling apart or pushing together the oculars.

To make diopter adjustments, one focuses first with the right eye only. Without touching the focusing knobs, diopter adjustments are then made on the left eye by turning the knurled **diopter adjustment ring** (figure 1.2) on the left ocular until a sharp image is seen. One should now be able to see sharp images with both eyes.

Resolution

It would appear that the magnification of a microscope is only limited by the magnifying power of a lens system. However, in reality the limit for most light microscopes is 1000×, which is set by an intrinsic property of lenses called **resolving power.** The resolving power of a lens is its ability to completely separate two objects in a microscopic field. The resolving power is given by the formula $d = 0.5\ \lambda/\text{NA}$. The limit of resolution, d, or the distance between the two objects, is a function of two properties: the wavelength of the light used to observe a specimen, λ, and a property of lenses called the **numerical aperture,** or NA. Numerical aperture is a mathematical expression that describes how the condenser lens concentrates and focuses the light rays from the light source. Its value is maximized when the light rays are focused into a cone of light that then passes through the specimen into the objective lens. However, because some light is refracted or bent as it passes from glass into air, the refracted light rays are lost, and as a result the

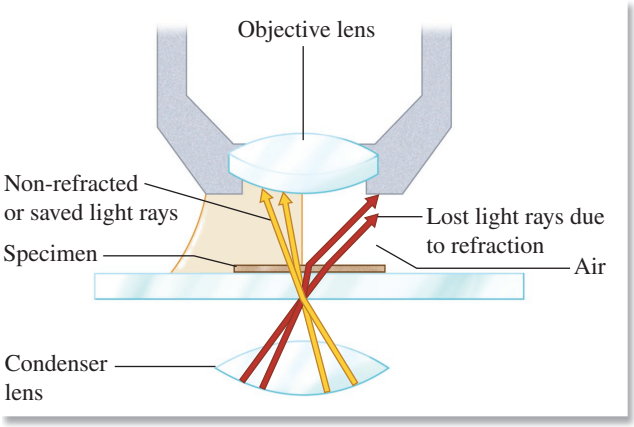


Figure 1.4 **Immersion oil, having the same refractive index as glass, prevents light loss due to refraction.**

numerical aperture is diminished (figure 1.4). The greater the loss of refracted light, the lower the numerical aperture. The final result is that the resolving power is greatly reduced.

For any light microscope, the limit of resolution is about 0.2 µm. This means that two objects closer than 0.2 µm would not be seen as two distinct objects. Because bacterial cells are about 1 µm, the cells can be resolved by the light microscope, but that is not the case for internal structures in bacterial cells that are smaller than 0.2 µm.

In order to maximize the resolving power from a lens system, the following should be considered:

- A **blue filter** should be placed over the light source because the shorter wavelength of the resulting light will provide maximum resolution.
- The condenser should be kept at the highest position that allows the maximum amount of light to enter the objective lens and therefore limit the amount of light lost due to refraction.
- The diaphragm should not be stopped down too much. While closing the diaphragm improves the contrast, it also reduces the numerical aperture.
- **Immersion oil** should be used between the slide and the 100× objective lens. This is a special oil that has the same refractive index as glass. When placed between the specimen and objective lens, the oil forms a continuous lens system that limits the loss of light due to refraction.

The bottom line is that for magnification to increase, resolution must also increase. Thus, a greater magnification cannot be achieved simply by adding a stronger ocular lens.

Lens Care

Keeping the lenses of your microscope clean is a constant concern. Unless all lenses are kept free of dust, oil, and other contaminants, they cannot achieve the degree of resolution that is intended. Consider the following suggestions for cleaning the various lens components:

Figure 1.5 **When oculars are removed for cleaning, cover the ocular opening with lens tissue. A blast from an air syringe or gas canister removes dust and lint.**

Cleaning Tissues Only lint-free, optically safe tissues should be used to clean lenses. Tissues free of abrasive grit fall in this category. Booklets of lens tissue are most widely used for this purpose. Although several types of boxed tissues are also safe, *use only the type of tissue that is recommended by your instructor* (figure 1.5).

Solvents Various liquids can be used for cleaning microscope lenses. Green soap with warm water works very well. Xylene is universally acceptable. Alcohol and acetone are also recommended, but often with some reservations. Acetone is a powerful solvent that could possibly dissolve the lens mounting cement in some objective lenses if it were used too liberally. When it is used it should be used sparingly. Your instructor will inform you as to what solvents can be used on the lenses of your microscope.

Oculars The best way to determine if your eyepiece is clean is to rotate it between the thumb and forefinger as you look through the microscope. A rotating pattern will be evidence of dirt.

If cleaning the top lens of the ocular with lens tissue fails to remove the debris, one should try cleaning the lower lens with lens tissue and blowing off any excess lint with an air syringe or gas canister. *Whenever the ocular is removed from the microscope, it is imperative that a piece of lens tissue be placed over the open end of the microscope as illustrated in figure 1.5.*

Objectives Objective lenses often become soiled by materials from slides or fingers. A piece of lens tissue moistened with green soap and water, or one of the acceptable solvents mentioned previously, will usually remove whatever is on the lens. Sometimes a cotton swab with a solvent will work better than lens tissue. At any time that the image on the slide is unclear or cloudy, assume at once that the objective you are using is soiled.

Condenser Dust often accumulates on the top surface of the condenser; thus, wiping it off occasionally with lens tissue is desirable.

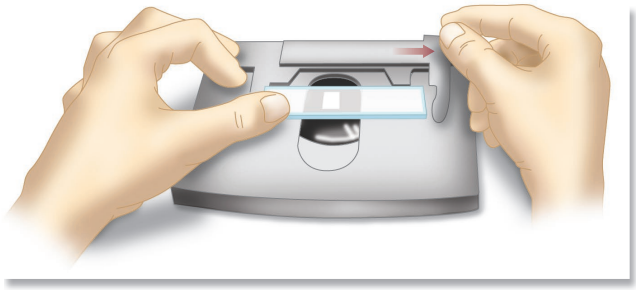


Figure 1.6 **The slide must be properly positioned as the retainer lever is moved to the right.**

Procedures

If your microscope has three objectives, you have three magnification options: (1) low-power, or 100× total magnification, (2) high-dry magnification, which is 400× total with a 40× objective, and (3) 1000× total magnification with a 100× oil immersion objective.

Whether you use the low-power objective or the oil immersion objective will depend on how much magnification is necessary. Generally speaking, however, it is best to start with the low-power objective and progress to the higher magnifications as your study progresses. Consider the following suggestions for setting up your microscope and making microscopic observations.

Low-Power Examination The main reason for starting with the low-power objective is to enable you to explore the slide to look for the object you are planning to study. Once you have found what you are looking for, you can proceed to higher magnifications. Use the following steps when exploring a slide with the low-power objective:

1. Position the slide on the stage with the material to be studied on the *upper* surface of the slide. Figure 1.6 illustrates how the slide must be held in place by the mechanical stage retainer lever.
2. Turn on the light source, using a *minimum* amount of voltage. If necessary, reposition the slide so that the stained material on the slide is in the *exact center* of the light source.
3. Check the condenser to see that it has been raised to its highest point.
4. If the low-power objective is not directly over the center of the stage, rotate it into position. Be sure that as you rotate the objective into position it clicks into its locked position.
5. Turn the coarse adjustment knob to lower the objective until it stops. A built-in stop will prevent the objective from touching the slide.
6. While looking down through the ocular (or oculars), bring the object into focus by turning the fine adjustment focusing knob. Don't readjust the coarse adjustment knob. If you are using a binocular microscope, it will also be necessary to adjust the interocular distance and diopter adjustment to match your eyes.
7. For optimal viewing, it is necessary to focus the condenser and adjust it for maximum illumination. This procedure should be performed each time the objective lens is changed. Raise the iris diaphragm to its highest position. Close the iris diaphragm until the edges of the diaphragm image appear fuzzy. Lower the condenser using its adjustment knob until the edges of the diaphragm are brought into sharp focus. You should now clearly see the sides of the diaphragm expand beyond the field of view. Refocus the specimen using the fine adjustment. Note that as you close the iris diaphragm to reduce the light intensity, the contrast improves and the depth of field increases. **Depth of field** is defined as the range of distance in front of and behind a focused image within which other objects will appear clear and sharply defined.
8. Once an image is visible, move the slide about to search out what you are looking for. The slide is moved by turning the knobs that move the mechanical stage.
9. Check the cleanliness of the ocular, using the procedure outlined earlier.
10. Once you have identified the structures to be studied and wish to increase the magnification, you may proceed to either high-dry or oil immersion magnification. However, before changing objectives, *be sure to center the object you wish to observe.*

High-Dry Examination To proceed from low-power to high-dry magnification, all that is necessary is to rotate the high-dry objective into position and open up the diaphragm somewhat. It may be necessary to make a minor adjustment with the fine adjustment knob to sharpen up the image, but *the coarse adjustment knob should not be touched.*

Good quality modern microscopes are usually both **parfocal** and **parcentral.** This means that the

image will remain both centered and in focus when changing from a lower-power to a higher-power objective lens.

When increasing the lighting, be sure to open up the diaphragm first instead of increasing the voltage on your lamp; the reason is that *lamp life is greatly extended when used at low voltage.* If the field is not bright enough after opening the diaphragm, feel free to increase the voltage. A final point: Keep the condenser at its highest point.

Oil Immersion Techniques The oil immersion lens derives its name from the fact that a special mineral oil is interposed between the specimen and the 100× objective lens. As stated previously, this reduces light refraction and maximizes the numerical aperture to improve the resolution. The use of oil in this way enhances the resolving power of the microscope. Figure 1.4 reveals this phenomenon.

With parfocal objectives one can go directly to oil immersion from either low-power or high-dry. On some microscopes, however, going from low-power to high-power and then to oil immersion is better. Once the microscope has been brought into focus at one magnification, the oil immersion lens can be rotated into position without fear of striking the slide.

Before rotating the oil immersion lens into position, however, a drop of immersion oil must be placed on the slide. An oil immersion lens should never be used without oil. Incidentally, if the oil appears cloudy, it should be discarded.

When using the oil immersion lens, more light is necessary to adequately visualize an image. Opening the diaphragm increases the resolving power of the microscope at higher magnifications. Thus, the iris diaphragm must be opened wider when using the oil immersion lens. Also, do not forget to refocus the condenser when moving from lower-power to higher-power objectives. Some microscopes also employ blue or green filters on the lamp housing to enhance resolving power.

Since the oil immersion lens will be used extensively in all bacteriological studies, it is of paramount importance that you learn how to use this lens properly. Using this lens takes a little practice due to the difficulties usually encountered in manipulating the lighting. It is important for all beginning students to appreciate that the working distance of a lens, the distance between the lens and microscope slide, decreases significantly as the magnification of the lens increases (table 1.1). Hence, the potential for damage to the oil immersion lens because of a collision with the microscope slide is very great. A final comment of importance: At the end of the laboratory period remove all immersion oil from the lens tip with lens tissue.

Table 1.1 **Relationship of Working Distance to Magnification**

LENS	MAGNIFICATION	FOCAL LENGTH (mm)	WORKING DISTANCE (mm)
Low-power	10×	16.0	7.7
High-dry	40×	4.0	0.3
Oil immersion	100×	1.8	0.12

Putting It Away

When you take a microscope from the cabinet at the beginning of the period, you expect it to be clean and in proper working condition. The next person to use the instrument after you have used it will expect the same consideration. A few moments of care at the end of the period will ensure these conditions. Check over the following list of items at the end of each period before you return the microscope to the cabinet.

1. Remove the slide from the stage.
2. If immersion oil has been used, wipe it off the lens and stage with lens tissue. Also, make sure that no immersion oil is on the 40× objective. This lens often becomes contaminated with oil as a result of mistakes made by beginning students. (Do not wipe oil off slides you wish to keep. Simply put them into a slide box and let the oil drain off.)
3. Rotate the low-power objective into position.
4. If the microscope has been inclined, return it to an erect position.
5. If the microscope has a built-in movable lamp, raise the lamp to its highest position.
6. If the microscope has a long attached electric cord, wrap it around the base.
7. Adjust the mechanical stage so that it does not project too far on either side.
8. Replace the dustcover.
9. If the microscope has a separate transformer, return it to its designated place.
10. Return the microscope to its correct place in the cabinet.

Laboratory Report

Before the microscope is to be used in the laboratory, answer all the questions in Laboratory Report 1. Preparation on your part prior to going to the laboratory will greatly facilitate your understanding. Your instructor may wish to collect this report at the *beginning of the period* on the first day that the microscope is to be used in class.

Laboratory Report 1

Student: _____

Date: _____ Section: _____

1 Brightfield Microscopy

A. Short-Answer Questions

1. Describe the position of your hands when carrying the microscope to and from your laboratory bench.

2. Differentiate between the limit of resolution of the typical light microscope and that of the unaided human eye.

3. (a) What two adjustments can be made to the condenser? (b) What effect do these adjustments have on the image?

4. Why are condenser adjustments generally preferred over the use of the light intensity control?

5. When using the oil immersion lens, what four procedures can be implemented to achieve the maximum resolution?

6. Why is it advisable to start first with the low-power lens when viewing a slide?

7. Why is it necessary to use oil in conjunction with the oil immersion lens and not with the other objectives?

8. What is the relationship between the working distance of an objective lens and its magnification power?