THIRTEENTH EDITION

Benson's

MICROBIOLOGICAL APPLICATIONS

Laboratory Manual in General Microbiology

Alfred Brown / Heidi Smith

SHORT VERSION

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Laboratory Manual in General Microbiology

Thirteenth Edition

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Emeritus Professor, Auburn University

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Mc Graw Hill Education

BENSON'S MICROBIOLOGICAL APPLICATIONS: LABORATORY MANUAL IN GENERAL MICROBIOLOGY, SHORT VERSION, THIRTEENTH EDITION

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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

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 in weed populations. My laboratory also investigated how the sulfonylurea herbicides inhibit acetolactate synthase, a crucial enzyme in the pathway for branched-chain amino acids. Most recently, I and my graduate students 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. The resulting discoloration caused by these bacteria on shingles has caused significant financial losses to the industry. My laboratory isolated various species of cyanobacteria involved in the problem and taxonomically characterized them. We also tested possible growth inhibitors that might be used in their control.

Teaching Dr. Brown 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. At present, he continues to work on this manual and travel extensively.

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.

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 member 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 has also served as the director of the Honors Program at the college for five years, working with a group of faculty to build the program from the ground up.

Student success is a strategic priority at FRCC and a personal passion of Heidi's, and she continually works to develop professionally in ways that help her do a better job of reaching this important goal. Throughout the past few years, Heidi has had the opportunity to collaborate with faculty all over the country in developing digital tools, such as LearnSmart, LearnSmart Labs, and Connect, to facilitate student learning and measure learning outcomes. This collaborative experience and these tools have revolutionized her approach to teaching and have dramatically affected student performance in her courses, especially microbiology hybrid courses where content is delivered partially online.

Heidi is an active member of the American Society for Microbiology and 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.

Contents

LEARNSMART LABS™ MICROBIOLOGY

Indicates a LearnSmart Lab™ activity is available for all or part of this exercise. For more information, visit mhhe.com/lslabsmicro.

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.

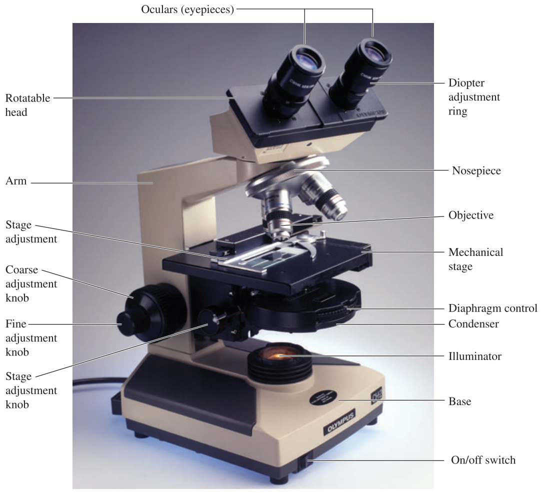


Figure 1.2 **The compound microscope.**
© Charles D. Winters/Science Source.

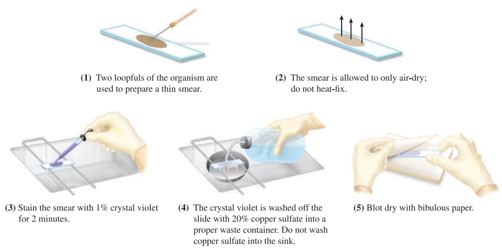
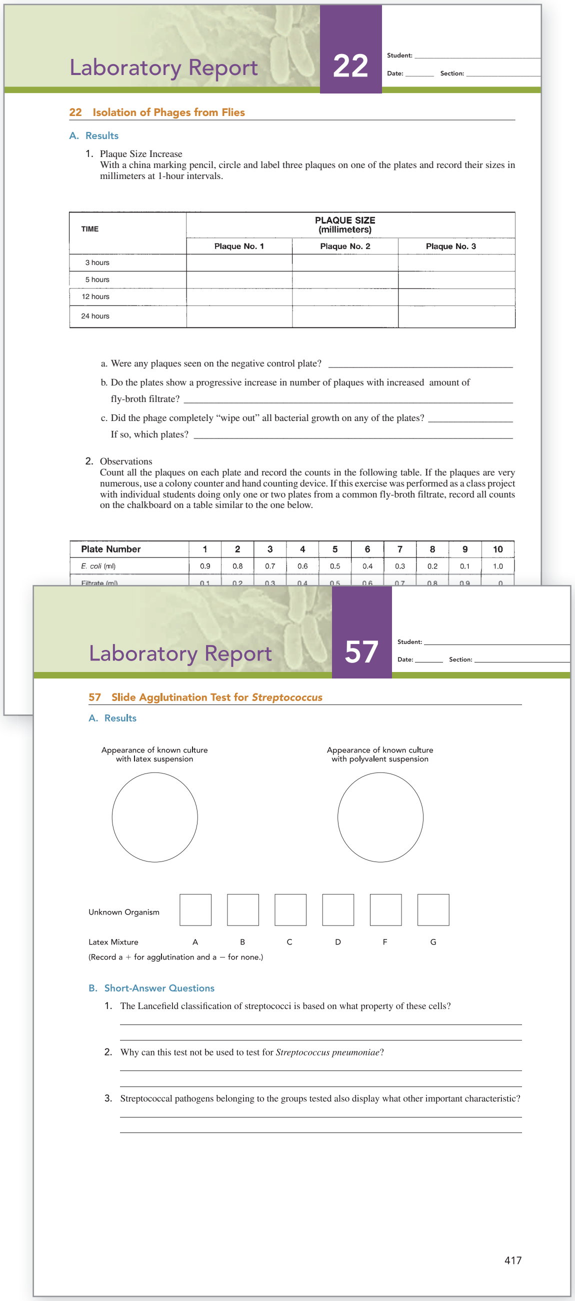


Figure 13.1 **Procedure for demonstration of a capsule.**



Laboratory Report 22

Student: ____________
Date: ______ Section: ____________

22 Isolation of Phages from Flies

A. Results

1. Plaque Size Increase
With a china marking pencil, 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, record all counts on the chalkboard on a table similar to the one below.

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

Laboratory Report 57

Student: ____________
Date: ______ Section: ____________

57 Slide Agglutination Test for *Streptococcus*

A. Results

Appearance of known culture with latex suspension

Appearance of known culture with polyvalent suspension

Unknown Organism

Latex Mixture A B C D F G

(Record a + for agglutination and a − for none.)

B. Short-Answer Questions

1. The Lancefield classification of streptococci is based on what property of these cells?

2. Why can this test not be used to test for *Streptococcus pneumoniae*?

3. Streptococcal pathogens belonging to the groups tested also display what other important characteristic?

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Laboratory Reports

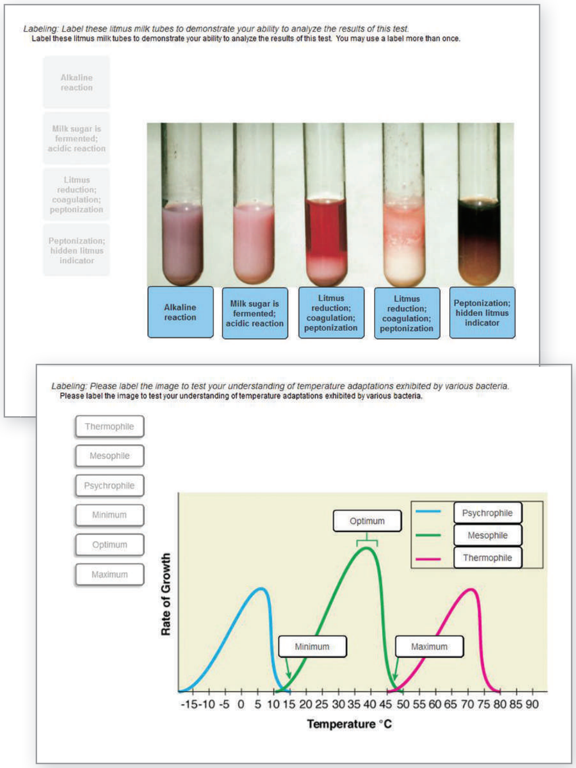
A Laboratory Report to be completed by students immediately follows most of the exercises. These Laboratory Reports are designed to guide and reinforce student learning and provide a convenient place for recording data. These reports include various types of review activities, tables for recoding observations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

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 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.



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™

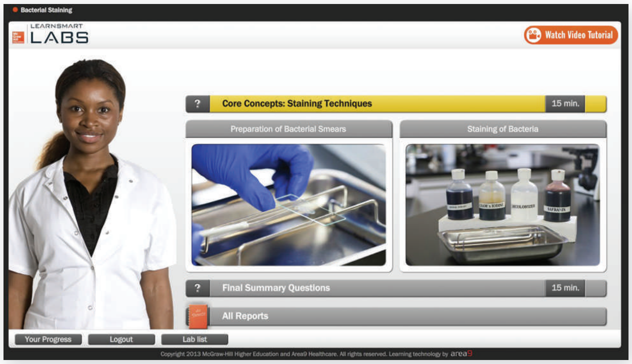
Fueled by LearnSmart—the most widely used and intelligent adaptive learning resource—LearnSmart Labs is a highly realistic and adaptive simulated lab experience that allows students to "do and think" like scientists.

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 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 to learn more.

Website

Visit the website at 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 laboratory manuals. This laboratory manual is available as an eBook at 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 to learn more.

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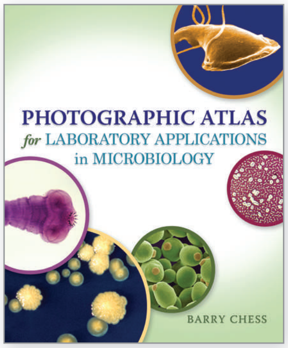
Student Resources



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 provide 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/cls 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 5, 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 6, 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 11, Simple Staining.
- The Fungi: Molds and Yeasts, Exercise 7, 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 10–16 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 14, 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 19, 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 21, Determination of a Bacteriophage Titer.

Part 7, Environmental Influences and Control of Microbial Growth

- Exercise 24, 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 30, Evaluation of Alcohol: Its Effectiveness as an Antiseptic.
- Antimicrobic Sensitivity Testing: The Kirby-Bauer Method, Exercise 31, 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 36, 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 37, Physiological Characteristics: Hydrolytic and Degradative Reactions, are new photos of starch, casein, and fat hydrolysis.
- Physiological Characteristics: Multiple Test Media, Exercise 38, 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 39, 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 11, Medical Microbiology

- Exercise 51, 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 52, includes a new photo of Gram stain of strep.
- Exercise 53, Gram-Negative Intestinal Pathogens, includes new photos of lactose fermenters on McConkey and Eosin methylene blue agar.
- A Synthetic Epidemic, Exercise 54, 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 12, Immunology and Serology

- Exercise 56, 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 Warenda Czura *Suffolk County Community College Eastern Campus*
Angela Edwards *Trident Technical College*
Mary Farone *Middle Tennessee State University*
Deborah Faurot *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*
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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*

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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 section 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, glanders, brucellosis, and tetanus, to name a few. Recent reports have documented laboratory-acquired cases in laboratory workers and health-care personnel involving *Bacillus anthracis, Bordetella pertussis, Brucella, Burkholderia pseudomallei, Campylobacter, Chlamydia,* and toxins from *Clostridium tetani, Clostridium botulinum,* and *Corynebacterium diphtheriae.* While we have a greater knowledge of these agents and antibiotics with which to treat them, safety and handling still remain primary issues.

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 and is provided by good microbiological technique and the use of appropriate safety equipment. 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 elements of laboratory design.

Biosafety Levels (BSL)

The recommended biosafety level(s) for handling microorganisms represent the potential of the agent to cause disease and the conditions under which the agent should be safely handled. The Centers for Disease Control classifies organisms into levels and sets guidelines for handling and safety measures required. These levels take into account many factors such as virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, and other factors. The recommended biosafety levels are as follows:

1. **BSL 1—**agents not known to cause disease in healthy adults; standard microbiological practices (SMP) apply; no safety equipment required; sinks required. Examples: *Bacillus subtilis, Micrococcus luteus.*
2. **BSL 2—**agents associated with human disease; standard microbiological practices apply plus limited access, biohazard signs, sharps precautions, and a biosafety manual required. Biosafety cabinet (BSC) used for aerosol/splash generating operations; lab coats, gloves, face protection required; contaminated waste is autoclaved.

All microorganisms used in the exercises in this manual are classified as BSL 1 or BSL 2. Examples: *Staphylococcus aureus, Streptococcus pyogenes.* **Note:** Although some of the organisms that students will culture and work with are classified as BSL 2, these organisms are 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.

3. **BSL 3—**indigenous/exotic agents that may have serious or lethal consequences and with a potential for aerosol transmission. BSL 2 practices plus controlled access; decontamination of all waste and lab clothing before laundering; determination of baseline antibody titers to agents; biosafety cabinets used for all specimen manipulations; respiratory protection used as needed; physical separation from access corridors; double door access; negative airflow into the lab; exhaust air not recirculated. Examples: *Mycobacterium tuberculosis* and vesicular stomatitis virus (VSV).
4. **BSL 4—**dangerous/exotic agents of a life-threatening nature or unknown risk of transmission; BSL 3 practices plus clothing change before entering the laboratory; shower required before leaving the lab; all materials decontaminated on

exit; positive pressure personnel suit required for entry; separated/isolated building; dedicated air supply/exhaust and decontamination systems. Examples: Ebola and Lassa viruses.

Each of the biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed and the documented or suspected routes of transmission of the infectious agents. Common to all biosafety levels are standard practices, especially aseptic technique. Refer to the Biosafety Levels table on page xv for a list of common organisms.

Standard Laboratory Rules and Practices

1. 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, the laboratory manual, and pen/pencil should be brought to the student work area.
2. Eating, drinking, chewing gum, and smoking are not allowed in the laboratory. Students must also avoid handling contact lenses or applying makeup while in the laboratory.
3. Safety equipment:
   a. Some labs will require that lab coats be worn in the laboratory at all times. Others may make this optional or not required. Lab coats can protect a student from contamination by microorganisms that he/she is working with and prevent 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. Lab coats, gloves, and safety equipment should not be worn outside of the laboratory unless properly decontaminated first.
   b. You may be required to wear gloves while performing the lab exercises. This is especially important if you have open wounds. They protect the hands against contamination by microorganisms and prevent the hands from coming in direct contact with stains and other reagents.
   c. Face protection/safety glasses may be required by some instructors while you are performing experiments. Safety glasses can prevent materials from coming in contact with the eyes. They must be worn especially when working with ultraviolet light to prevent eye damage because they block out UV rays. If procedures involve the potential for splash/aerosols, face protection should be worn.
   d. Know the location of eye wash and shower stations in the event of an accident that requires the use of this equipment. Also know the location of first aid kits.
4. 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.
5. 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.
6. 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. Avoid contamination of the work surface by not placing contaminated pipettes, loops/needles, or swabs on the work surface. Dispose of contaminated paper towels used for swabbing in the biohazard container.
7. Use extreme caution when working with open flames. The flame on a Bunsen burner is often difficult to see when not in use. 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.**
8. Any cuts or injuries on the hands must be covered with band-aids to prevent contamination. If you injure or cut yourself during the laboratory, notify the laboratory instructor immediately.
9. Pipetting by mouth is prohibited in the lab. All pipetting must be performed with pipette aids. Be especially careful when inserting glass pipettes into pipette aids as the pipette can break and cause a serious injury.
10. Know the location of exits and fire extinguishers in the laboratory.
11. Most importantly, read the exercise and understand the laboratory protocol before coming to laboratory. In this way you will be familiar with potential hazards in the exercise.
12. When working with microfuges, be familiar with their safe operation and make sure that all microfuge tubes are securely capped before centrifuging.
13. When working with electrophoresis equipment, follow the directions carefully to avoid electric shock.
14. If you have any allergies or medical conditions that might be complicated by participating in the laboratory, inform the instructor. Women who are pregnant should discuss the matter of enrolling in

the lab with their family physician and the laboratory instructor.

15. Unless directed to do so, do not subculture any unknown organisms isolated from the environment as they could be potential pathogens.
16. Avoid handling personal items such as cell phones, calculators, and cosmetics while performing the day's exercise.
17. 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.
18. Avoid wearing dangling jewelry to lab.

Disposal of Biological Wastes

Dispose of all contaminated materials properly and in the appropriate containers:

1. Biohazard containers—biohazard containers are to be lined with clear autoclave bags; disposable petri 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.
2. Sharps containers—sharps, slides, coverslips, broken glass, disposable pipettes, and Pasteur pipettes should be discarded in these containers. If instructed to do so, you can discard contaminated swabs, wooden sticks, and microfuge tubes in the sharps containers.
3. Discard shelves, carts, bins, etc.—contaminated culture tubes and glassware used to store media and other glassware should be placed in these areas for decontamination and washing.
4. 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.

Discard other materials as directed by your instructor. This may involve placing materials such as slides contaminated with blood in disinfectant baths before these materials can be discarded.

Emergencies

Surface Contamination

1. Report all spills immediately to the laboratory instructor.
2. Cover the spill with paper towels and saturate the paper towels with disinfectant.
3. Allow the disinfectant to act for at least 20 minutes.
4. Remove any glass or solid material with forceps or scoop and discard the waste in an appropriate manner.

Personnel Contamination

1. Notify lab instructor.
2. Clean exposed area with soap/water, eye wash (eyes) or saline (mouth).
3. Apply first aid.

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* *Enterococcus faecalis* *Micrococcus luteus* *Neisseria sicca* *Proteus vulgaris* *Pseudomonas aeruginosa* *Staphylococcus epidermidis* *Staphylococcus saprophyticus*
BSL 2	Poses a moderate risk to healthy adults; unlikely to spread throughout community; effective treatment readily available.	*Enterobacter aerogenes* *Escherichia coli* *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

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	49
Bacillus megaterium ATCC 14581	Positive rod	Soil, water	1	11, 12, 14, 15, 28, 48
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	1	9
Citrobacter freundii ATCC 8090	Negative rod	Humans, animals, soil water; sewage opportunistic pathogen	1	53
Clostridium beijerinckii ATCC 25752	Positive rod	Soil	1	24
Clostridium sporogenes ATCC 3584	Positive rod	Soil, animal feces	1	24, 49
Corynebacterium xerosis ATCC 373	Positive rods, club-shaped	Conjunctiva, skin	1	11
Enterobacter aerogenes ATCC 13048	Negative rods	Feces of humans and animals	2	24, 36, 39, 45
Enterococcus faecalis ATCC 19433	Positive cocci in pairs, short chains	Water, sewage, soil, dairy products	2	24, 39, 52, 57
Enterococcus faecium ATCC 19434	Positive cocci in pairs, short chains	Feces of humans and animals	2	52, 57
Escherichia coli ATCC 11775	Negative rods	Sewage, intestinal tract of warm-blooded animals	2	8, 9, 14, 19, 21, 22, 24, 25, 26, 27, 29, 31, 36, 37, 38, 39, 45, 48, 49
Geobacillus stearothermophilus ATCC 12980	Gram-positive rods	Soil, spoiled food	1	25, 49
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

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

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Lactococcus lactis ATCC 19435	Positive cocci in chains	Milk and milk products	1	11
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	1	17, 31, 37, 38, 39, 53
Pseudomonas aeruginosa ATCC 10145	Negative rods	Soil and water; opportunistic pathogen in humans	1	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 700720D-5	Negative rods	Most frequent agent of *Salmonella* gastroenteritis in humans	2	39, 53, 55
Serratia marcescens ATCC 13880	Negative rods	Opportunistic pathogen in humans	1	9, 25, 39, 54
Shigella flexneri ATCC 29903	Negative rods	Pathogen of humans	2	53
Staphylococcus aureus ATCC 12600	Positive cocci, irregular clusters	Skin, nose, GI tract of humans, pathogen	2	9, 11, 12, 14, 16, 23, 24, 26, 27, 28, 29,31, 32, 36, 37, 38, 39, 48, 51, 52, 56
Staphylococcus epidermidis ATCC 14990	Positive cocci that occur in pairs and tetrads	Human skin, animals; opportunistic pathogen	1	39, 51
Staphylococcus saprophyticus ATCC 15305	Positive cocci that occur singly and in pairs	Human skin; opportunistic pathogen in the urinary tract	1	51
Streptococcus agalactiae ATCC 13813	Positive cocci; occurs in long chains	Upper respiratory and vaginal tract of humans, cattle; pathogen	2	52, 57
Streptococcus bovis ATCC 33317	Positive cocci; pairs and chains	Cattle, sheep, pigs; occasional pathogen in humans	2	52, 57
Streptococcus dysagalactiae subsp. equisimilis ATCC 43078	Positive cocci in chains	Mastitis in cattle	2	52

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	52
Streptococcus mutans ATCC 25175D-5	Positive cocci in pairs and chains	Tooth surface of humans, causes dental caries	2	52
Streptococcus pneumoniae ATCC 33400D-5	Positive cocci in pairs	Human pathogen	2	52
Streptococcus pyogenes ATCC 12344	Positive cocci in chains	Human respiratory tract; pathogen	2	52, 57
Streptococcus salivarius ATCC 19258	Positive cocci in short and long chains	Tongue and saliva	2	52
Thermoanaerobacterium thermosaccharolyticum ATCC 7956	Negative rods; single cells or pairs	Soil, spoiled canned foods	1	49

PART 1

Microscopy

Although there are many kinds of microscopes available to the microbiologist today, only four types will be described here for our use: the brightfield, darkfield, phase-contrast, and fluorescence 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.

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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 five 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.

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MICROBIOLOGY

EXERCISE

1

Brightfield Microscopy

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.

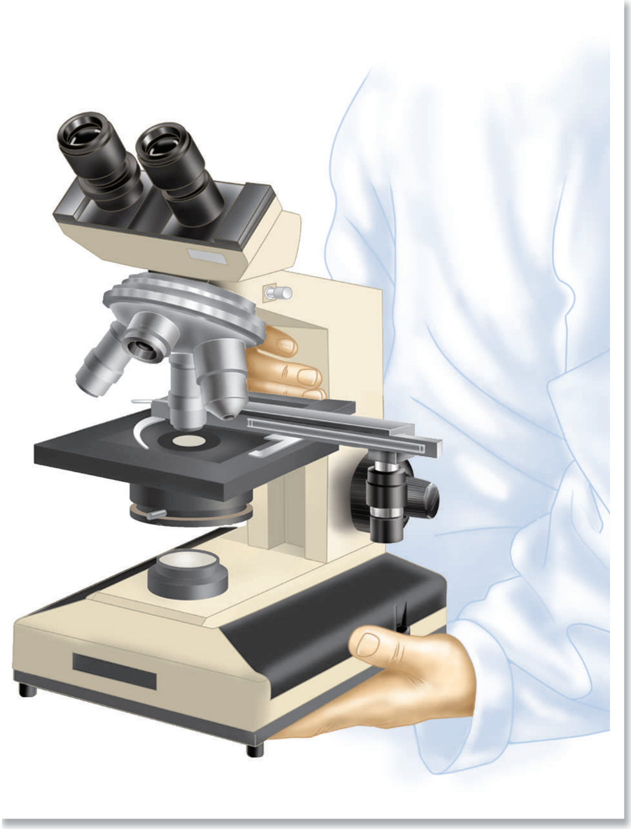

Figure 1.1 **The microscope should be held firmly with both hands while being carried.**

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.*

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 on page 6.

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.

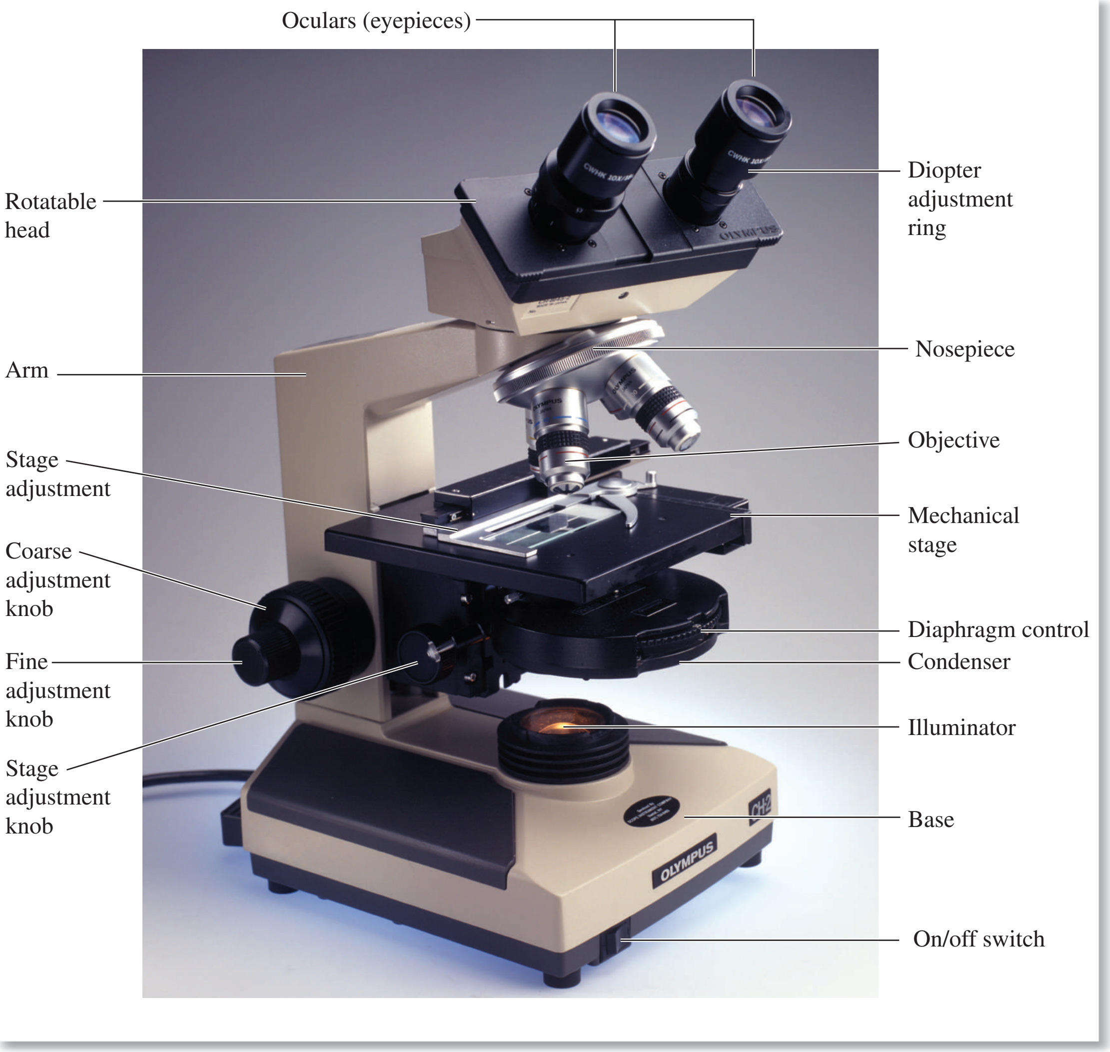

Figure 1.2 **The compound microscope.**

Components

Before we discuss the procedures for using a microscope, let's identify the principal parts of the instrument as illustrated in figure 1.2.

Framework All microscopes have a basic frame structure, which includes the **arm** and **base.** To this framework all other parts are attached. On many of the older microscopes the base is not rigidly attached to the arm as is the case in figure 1.2; instead, a pivot point is present that enables one to tilt the arm backward to adjust the eye point height.

Stage The horizontal platform that supports the microscope slide is called the **stage.** Note that it has a clamping device, the **stage adjustment,** which is used for holding and moving the slide around on the stage. Note, also, the location of the **stage adjustment knobs** in figure 1.2.

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 CX41, 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.

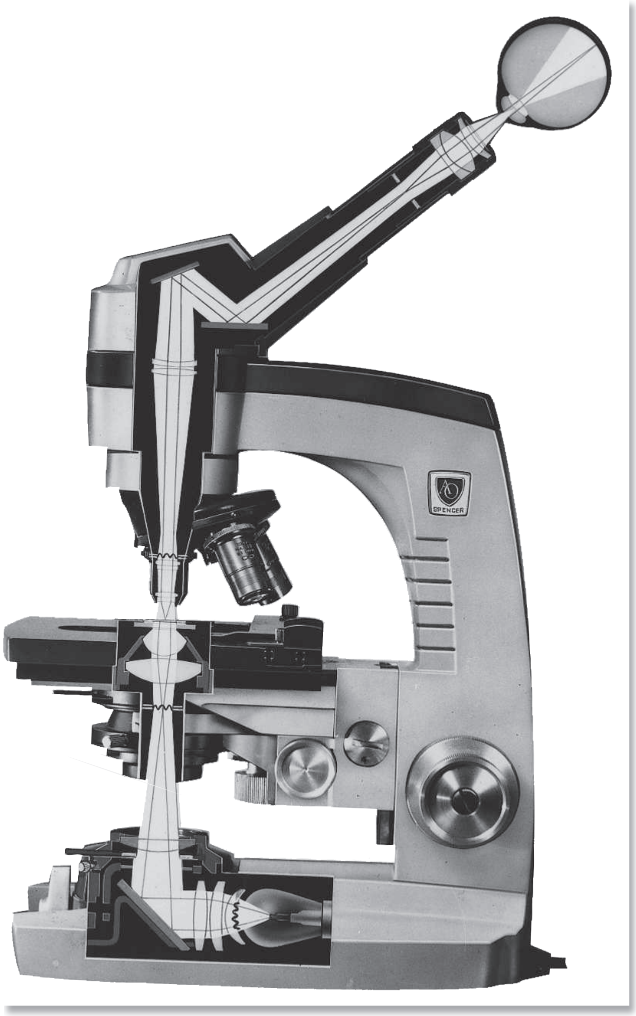

Figure 1.3 **The light pathway of a microscope.**

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.

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 CX41 microscope in figure 1.2, the diaphragm is controlled by turning a knurled ring. On some microscopes, a diaphragm lever is present. Figure 1.3 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

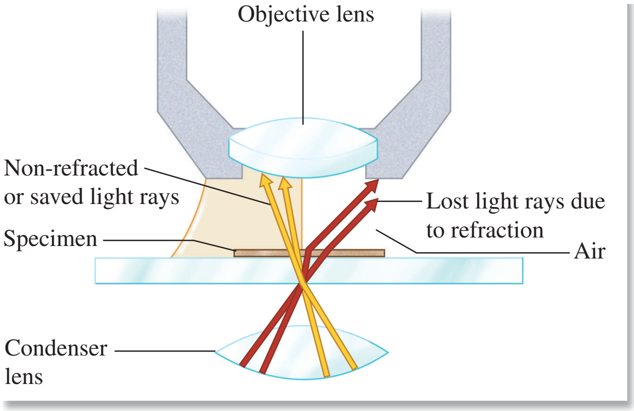

Figure 1.4 **Immersion oil, having the same refractive index as glass, prevents light loss due to refraction.**

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.**

lost, and as a result the 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:

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 on page 6, 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.

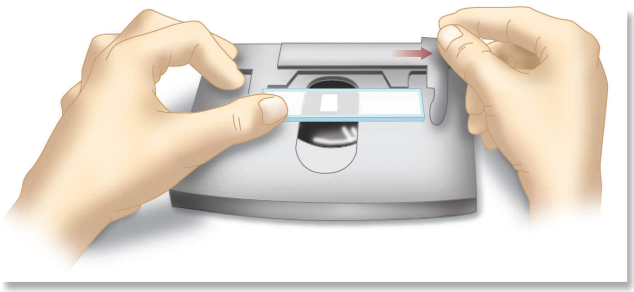

Figure 1.6 **The slide must be properly positioned as the retainer lever is moved to the right.**

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 on 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?