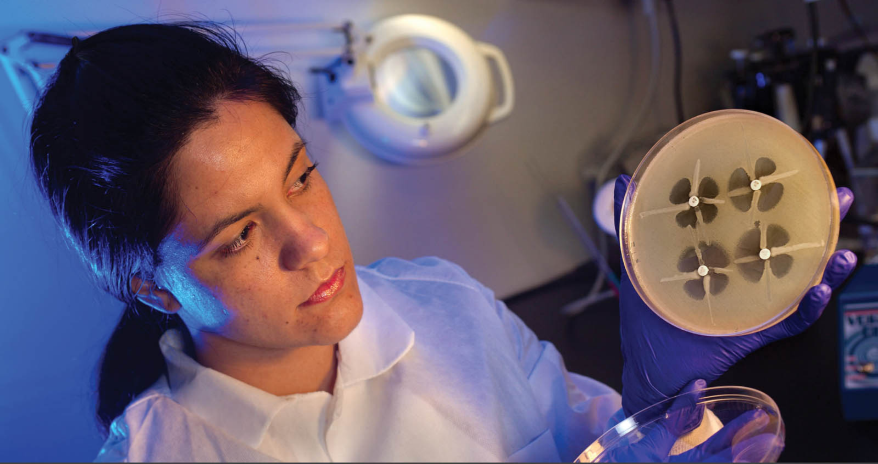

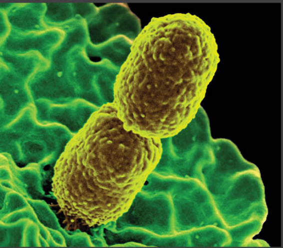

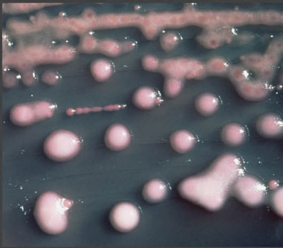

Laboratory Applications in MICROBIOLOGY

Fourth Edition

A Case Study Approach

Mc Graw Hill Education

BARRY CHESS

Laboratory Applications in Microbiology

A CASE STUDY APPROACH

Fourth Edition

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McGraw Hill Education

LABORATORY APPLICATIONS IN MICROBIOLOGY: A CASE STUDY APPROACH, FOURTH EDITION

This book is printed onacid-free paper.

1 2 3 4 5 6 7 8 9 LMN 21 20 19 18

ISBN 978-1-259-70522-9 (bound edition)
MHID 1-259-70522-6 (bound edition)
ISBN 978-1-260-41877-4 (loose-leaf edition)
MHID 1-260-41877-4 (loose-leaf edition)

Portfolio Manager: *Marija Magner*
Product Developers: *Rose Koos, Lora Neyens, and Darlene M. Schueller*
Marketing Manager: *Valerie L. Kramer*
Content Project Managers: *Becca Gill*
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Design: *Matt Backhaus*
Content Licensing Specialists: *Lorraine Buczek*
Cover Image: ©*CDC/James Gathany (CDC Researcher);* ©*CDC/National Institute of Allergy and Infectious Diseases (colorized SEM);* ©*McGraw-Hill Education, Lisa Burgess, photographer (pneumoniae capsule);* ©*Courtesy of the CDC (pneumoniae growing)*
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Preface

My students laugh at me.

Why? I typically begin a class by saying "the coolest thing happened in…" and relate the latest Ebola scare, botulism outbreak, or measles epidemic, growing more animated with each news headline. Okay, so maybe "cool" isn't the best word to describe these incidents, but I get excited when microbiology becomes a topic in the news—a flu outbreak in a small town or *E. coli* hitting a fast food chain. And as much as my students—and let's face it, most people—find disease outbreaks an odd thing to get excited about, microbiologists, microbiology teachers, nurses, doctors, and health officials of all stripes perk up their ears when they hear these stories.

One of the best things about living in the twenty-first century is that few of us know someone who contracted polio, died of botulism, or wasted away from tuberculosis. On the flip side, vaccination rates have fallen because no one has seen a family member suffer through measles or mumps. We have in some ways become victims of our own success. Disinfection of water, the appropriate use of antibiotics, and routine monitoring and testing of the food supply have turned much of microbiology teaching into something of a history class—"This used to happen until…." But the microbes have not gone away, and the very best way to emphasize that point is to mention the news story about a scientist who dies of the plague or a pair of roommates who contract botulism from pesto sauce they bought at the farmer's market. As microbiologists, we recognize the story in our Twitter feed or the blurb on the radio as a living example of microbiology's impact on our daily lives, and we share that with our students. Unfortunately, news stories do not always adhere to our syllabi, and many of these cool teaching moments go unexploited.

For Whom Is This Lab Manual Written?

Written for students entering the allied health fields, *Laboratory Applications in Microbiology: A Case Study Approach*, is designed to use real-life examples, or case studies, as the basis for exercises in the laboratory. Throughout the past few years, the number of lecture texts utilizing case studies has grown rapidly, and for good reason—case studies work! This book is a lab manual focusing on this means of instruction, an approach particularly applicable to the microbiology laboratory. All the microbiological theory in the world means little if students cannot understand the importance of a Gram stain, antibiogram, or other laboratory procedure.

What Sets This Lab Manual Apart?

This book was created to make the microbiology lab a more valuable experience by reconnecting the *what* and *how* of microbiology with the sometimes forgotten *why*. Although Latin names, complex media, and complicated assays will always be a part of the curriculum, the context of each exercise has been expanded so the reason for completing a specific task will be clear from the outset. Every sentence was written and each photograph chosen to accomplish this goal, and the result is a laboratory manual like nothing else in the field.

You'll notice a number of changes to the book, including:

Metacognition

Metacognition is often defined as thinking about thinking. To write a lab manual and not be concerned with how students are using it to learn would be irresponsible. Many references are available that accurately describe procedures, which are great tools for the accomplished microbiologist. In contrast, this book has been designed to help **create** accomplished microbiologists. Every exercise has been structured from the bottom up, scaffolding knowledge so students **understand** the goals of an exercise, **anticipate** errors, **acquire** the skills needed for success, and eventually **master** the topic at hand.

Each laboratory activity begins with a series of **Remember**, **Understand, Apply** questions, meant to ensure students have the requisite knowledge to **begin** an exercise. I think you'll agree that knowing why a particular test is performed, what is required to complete the test, and the appearance of both a negative and positive result is exactly what we'd like a student to know before beginning an activity. At the conclusion of the exercise, the student will encounter a set of **Analyze, Evaluate, Create** questions, meant to reinforce their facility with the topic at hand. Asking them to interpret unfamiliar outcomes, examine results in a different context, or troubleshoot problems; these questions help students make the critical leap from apprentice to microbiologist.

Case Studies

The first 40 exercises include cases taken from the scientific literature or mass media. The techniques, media, and observational tools introduced in each exercise help students solve the issues presented in the case, which drives home the relevance of microbiology and hones critical thinking skills. Evidence has shown that the use of case studies **boosts learning**, develops **critical thinking** skills, increases student **retention** and **success,** and even reduces the incidence of academic dishonesty. Simply put, students learn more, learn faster, and retain more with case studies than with traditional instruction methods. Although this seems obvious to those of us who cannot wait to share the day's news story with our class, the results are backed up by empirical evidence. In one study focused on instructors who use cases, 97 percent reported that students who were taught with cases learned new ways to think about an issue; 95 percent reported that students took a more active part in the learning process; and 92 percent reported that students were more engaged in classes. Truly remarkable numbers.

Photographs

Microbiology is certainly one of the most visual of the sciences. From the beginning, we are concerned with pink versus purple, red versus white blood cells, and how to tell the difference between the egg of a flatworm and that of a roundworm. To that end we have photographs. Hundreds of photographs that explain difficult concepts. By illustrating the answers to very basic questions: "What does a mold look like?" "Is my endospore stain positive?" or "Is that an oocyst?" students can **evaluate their work** with the eye of someone who has seen these things before. Exercises include photographs both of those things students are likely to see (spirogyra in a pond water sample) and those that are thankfully more rare (beta hemolytic streptococci, colonies of *Bacillus anthracis*). Throughout the manual, you'll notice more than 150 new photographs. Most are completely new to the manual, while others were found in the photo atlas and have since been moved to be more accessible (because no one likes flipping pages). These new photographs were chosen because they display innovative techniques, alternative methods, or simply because each was better than the photograph it replaced. Every photograph was selected with only one goal in mind: to make microbiology more understandable to the student.

There's More to the Story

There's More to the Story serves as a jumping off point for students who want to go the extra mile. Broadly written, these 10 addendums ask students to take the exercise they've just completed to the next step. After Exercise 17 (Lethal Effects of Ultraviolet Light) for example, students are encouraged to study the effects of environmental UV radiation on bacterial populations. Subsequent to an exercise on algae (Exercise 3), students have a chance to receive training and become volunteer researchers for the Phytoplankton Monitoring Network. Following an exercise on milk spoilage (Exercise 34), students can make—and study—their own fermented food. Whether used as extra credit, individual exercises, or even independent study projects, There's More to the Story requires students to do **research**, generate a **protocol**, and prove or disprove a **hypothesis**; in other words, act like the scientists we are training them to be.

Tech Tips

New to this edition, Tech Tips (and that is the last time you will hear about them by name, I had to call them something) are, very simply, the things we say to our class every day. They are the knowledge one accumulates after doing (or watching others do) something 10,000 times. They get neither a paragraph nor a number, just a note, if you will, inserted in the text, and are meant only to pass along an occasional quick hint.

Flip Your Classroom!

It's always good when a great idea gets a name. Hardly a new concept, "flipping" refers to students accessing basic information before coming to class, leaving more time during class for **problem solving**, **collaborative learning**, and the development of higher-order **critical thinking** skills. From the beginning, *Laboratory Applications in Microbiology* has been designed for the flipped classroom. **Student learning outcomes**, extensive laboratory introductions, and pre-lab questions all combine to let students begin their learning at home, maximizing the scarce time available in the lab and freeing teachers to spend more time teaching.

Changes to the Fourth Edition

As time passes, the microbes we study, and the manner in which we study them, evolve in concert with subjects both microbiological (biofilms, antibiotic resistance) and technological (serological testing, DNA profiling), becoming more important to a proper understanding of microbiology. With that in mind, you'll notice changes to the fourth edition, including four new labs (**Helminths**, **Latex Agglutination of Staphylococci**, **Biofilms**, and **Chromogenic Agar**), new organizational hierarchy for some groups of organisms, new techniques, and many, many new case studies. Once again, the only question that was asked was "Will this change result in better student understanding?" Major changes to the manual include the tips previously discussed, along with the addition of new questions to every exercise, including an entirely new set of **Remember**, **Understand**, **Apply** questions for each activity. A more specific list of changes includes:

Exercise 1: Safety Considerations in the Microbiology Laboratory

- The lab now contains an active learning exercise to familiarize students with safety equipment in the lab.
- Two new photographs were added.
- New case study has been added concerning a researcher infected with an attenuated strain of plague due to an unknown medical condition.
- The case concerning the UCLA student who died as a result of inadequate safety training has been updated.
- A new case study on a 2017 *Salmonella* outbreak traced to college laboratories has been added.

Exercise 3: A Survey of Protists

- A completely reworked lab now presents protists as a single group in accordance with the most contemporary taxonomic theories.
- The laboratory exercise now includes more than 50 new photographs.
- Slime molds and cyanobacteria—both discussion and photographs—have been incorporated into the exercise.

Exercise 4: A Survey of Fungi

- Fungi are organized into five groups according to the latest scientific understanding.
- The lab contains more than two dozen new photographs.

Exercise 5: A Survey of Parasitic Worms

- Parasitic helminths are presented as a completely new laboratory exercise.
- The exercise includes 20 new photographs and 2 new case studies centering on ingestion of parasitic worms.

Exercise 6: Ubiquity of Microorganisms

- The lab includes entirely new pre-lab questions, along with an expanded introduction.
- A new case study on a mucormycosis outbreak at the University of Pennsylvania Medical Center has been added.

Exercise 7: Aseptic and Pure Culture Techniques

- A new case study concerning typhoid fever in Colorado has been added.

Exercise 8: Simple Staining, Negative Staining, and Gram Staining

- A new staining gallery displays over a dozen photographs of stained bacterial cells.
- The case study concerning listeriosis associated with cantaloupe has been updated to reflect the latest legal ramifications of the case.

Exercise 12: Viable Plate Count

- A new case on an outbreak of *Campylobacter jejuni* infections associated with raw milk in Utah has been added.
- A new discussion of dilution and dilution factors has been added to the lab.

Exercise 13: Cultivation of Anaerobes

- A new case study involving botulism at a church picnic in Ohio has been added.

Exercise 15: pH and Microbial Growth

- A new case study concerning botulism from pesto sauce bought from a farmer's market has been added.

Exercise 16: Effects of Osmotic Pressure on Bacterial Growth

- A new case study involving *Vibrio vulnificus* infection of a recent tattoo has been added.

Exercise 19: Effectiveness of Hand Scrubbing

- A There's More to the Story activity, featuring the *iScrub lite* handwashing app to track hand hygiene, has been added.

Exercise 20: Antimicrobic Sensitivity Testing: Kirby-Bauer, Tube Dilution, and ETEST® Methods

- A new case study involving Carbapenem-resistant *Klebsiella pneumoniae* has been added.

Exercise 21: Simulated Epidemic

- A new case study involving a measles outbreak among the Somali community in Minnesota has been added.

Exercise 22: Morbidity and Mortality Weekly Report

- A new case study involving the use of social media to track outbreaks of food poisoning in Chicago, New York, and Las Vegas has been added.

Exercise 23: Bacterial Transformation

- A new case study involving the fourteenth documented case of VRSA has been added.

Exercise 24: The Ames Test

- A new case study involving Zika virus and the safety of chemicals used to repel the *Aedes* mosquito that serves as a vector for the virus has been added.

Exercise 26: DNA Profiling

- The case study concerning *Salmonella* infections associated with peanuts has been updated to include the latest legal ramifications for those involved.

Exercise 27: Blood Typing

- The statistics for hemolytic transfusion reactions have been updated.

Exercise 28: Rapid Identification of *Staphylococcus aureus* Using Latex Agglutination Testing

- An entirely new exercise focused on the use of rapid, immunologically based procedures has been added.
- A new case study concerning MRSA infections linked to reuse of single-dose drug vials is included in the exercise.

Exercise 29: Slide Agglutination

- A new case study concerning a *Salmonella* outbreak linked to environmental exposure has been added.

Exercise 30: Enzyme-Linked Immunosorbent Assay (ELISA)

- A new case study involving viral meningoencephalitis linked to a rodent infestation in Minnesota has been added.

Exercise 31: Biofilm Culture and Examination

- A new exercise focused on the importance of biofilm formation has been added.

- A new case study concerning *Mycobacterium abscessus* Infections Among Patients of Pediatric Dentistry Practices in Georgia and California has been added to the exercise.

Exercise 32: Measures of Water Quality: Most Probable Number Procedure

- A new case study concerning the appearance of *E. coli* in the water supply serving Walker County, Georgia, has been added.

Exercise 33: Measures of Water Quality: Membrane Filtration Method

- A new case study concerning water quality on aircraft is now part of the exercise.

Exercise 34: Measures of Milk Quality: Methylene Blue Reductase Test

- A new case study concerning West Virginia legislators becoming ill after consuming raw milk has been added.

Exercise 35: Bacterial Counts of Food

- A new case study which follows three very different incidents of food poisoning has been added.

Exercise 36: Isolation and Identification of Staphylococci

- A new case study which follows news reports of an NFL player battling a staph infection has been added.

Exercise 37: Isolation and Identification of Streptococci

- A new case study centered on a late case of Group B streptococcus related to a consumption of dehydrated placenta has been added.

Exercise 38: Epidemiology of Gastrointestinal Illness: Differentiation of Enterobacteriaceae

- Three new case studies, each of which examines an outbreak caused by a different species of bacteria is now part of the exercise.

Exercise 39: Differential White Blood Cell Count

- A new case study concerning trichinellosis linked to consumption of walrus meat has been added.

Exercise 49: CHROMagar Orientation Medium

- A new exercise covering the use of chromogenic media has been added.
- Several new photographs of bacterial growing on chromogenic agar are part of the exercise.

Exercise 57: Blood Agar

- A new photograph of β-hemolysis has been added to the exercise.

Exercise 80: Casease Test

- A new photograph of casein hydrolysis has been added to the exercise.

Exercise 90: Viable Plate Count

- Explanations and equations for calculating dilution factors and original cell densities have been clarified.

Exercise 91: Direct Cell Count

- Explanations and equations for calculating dilution factors and original cell densities have been clarified.

Progression of Exercises Promotes Active Learning

Material in each of the first 40 exercises has been carefully organized so that students develop a solid intellectual base, beginning with a particular technique, mastering it, and then applying this new knowledge to a case study. Immediately following the introductory material, pre-lab questions help students to focus on the important aspects of a technique, developing a framework for what they will need to do **prior to the lab,** many of which require two or three periods. Between the multiday labs, questions are posed to ensure that the students understand **what** they have just done, the **results** they should expect, and the **significance** of those results. Post-lab questions require applying the knowledge gained from the exercise to answer more thought-provoking questions about the techniques they have just studied. Each of the first 40 exercises concludes with a case study, a real-life situation in which the technique just mastered plays a starring role. Case study questions, generally higher-order thought questions, challenge students to apply the information they've learned to other situations. In a quarter of the exercises, open-ended topics for study are featured (There's More to the Story . . .) that allow students to move beyond the everyday and become true researchers.

While the first 40 exercises focus on case studies, the **why** of microbiology, the **how** of the subject has not been forgotten. The final 51 exercises serve as a thorough compendium of common microbiological methods. These exercises are presented in such a way that students will develop critical thinking skills simply by deciding on a particular course of action. All similar techniques, such as selective and differential media or biochemical tests, are grouped together, and each exercise begins with student learning objectives and a brief overview. By reviewing the overview, a student may select an appropriate test, media, or staining technique from the many available, ensuring that they have decided not only what information they need, but how to go about getting it. Written to clearly guide students while also pointing out the importance of a particular technique, this portion of the manual provides detailed, well-illustrated procedures that stand by themselves or can be used in conjunction with the case studies in the front of the book. This is particularly helpful when undertaking unknowns, as each student's unknown culture will require a unique set of procedures for complete identification. A data sheet in exercise 40 provides a single location for students to record their test results, reinforcing the importance of record keeping in the laboratory.

Extensive Flowcharts for Bacterial Identification

Exercise 40 introduces the concept of bacterial identification, using a case study recounting the recognition of *Legionella pneumophila* as the causative agent of Legionnaires' disease. Within this exercise, 31 flowcharts are used to help identify bacterial unknowns commonly seen in the microbiology laboratory, a far more extensive collection than the one or two found in most manuals. This exercise also serves as an introduction to the techniques section of the manual, allowing students to quickly decide which diagnostic techniques are applicable to their particular unknown culture.

A Self-Contained Resource for the Microbiology Laboratory

In the workplace, allied health professionals are expected to evaluate a situation and find a solution using whatever resources are available to them. This book serves as a self-contained resource, with everything a student needs to solve a problem in the microbiology laboratory. A **glossary** provides definitions of all microbiological terms used in the book, a rarity in the field. **Appendices** contain the formula of every medium and reagent used, in addition to **tutorials** covering universal techniques, like the use of pipettes and spectrophotometers as well as the preparation of media. Each exercise also includes a **link to applicable websites,** such as the CDC homepage for each pathogenic microorganism encountered. In short, this book will help students develop the ability to solve problems.

Personalize Your Lab

McGraw-Hill Create™ is a self-service website that allows you to create custom course materials using McGraw-Hill Education's comprehensive, cross-disciplinary content and digital products.

Acknowledgments

Occasionally, one of my students asks, "how do you write a book?" While my answer usually runs toward some self-important nonsense like "Well, first you have a vision of what you want to accomplish…," the real answer is "Find of group of very smart people who are experts at what they do." Without the help and support of a whole bunch of people, I'd still be searching for my vision. The first thank you, as always, goes to my students, who are experts…at being students. They know—and let me know—what works and what doesn't. I know you didn't sign up to be test subjects for every idea that pops into my head, but there is no way I could do this without your good-natured feedback. Please know that you have helped create a better book. In the lab at Pasadena City College, a great number of people have supplied ideas, critiques, and criticisms that have helped shape this book. Special thanks go to Jessica Igoe and Sonya Valentine, two unbelievable teachers and microbiologists from whom I learn something new each semester; John Stantzos (who never uses the phrase "it's Greek to me," because he knows Greek—how cool is that?); and Ray Burke. (Any really bad jokes in this book are probably Ray's.) Of course, absolutely nothing happens in the lab without the support of Mary Timmer, laboratory technician of the gods.

The second group of very smart people—who do know how to make a book—are the talented professionals at McGraw-Hill Education. Undying gratitude to Senior Product Developer, Darlene Schueller, and Senior Portfolio Manager, Marija Magner. There is no way to adequately thank anyone who has been assigned to either develop or manage me, so I will inadequately thank them. Further thanks to Marketing Manager Valerie Kramer, Content Project Manager Becca Gill (again, managing me…), Designer Matt Backhaus, and Content Licensing Specialist Lorraine Buczek. The people I've had a chance to work with at McGraw-Hill Education are talented professionals, and I count myself lucky to work with them.

More very smart people—my wife and kids—who put up with a lot, and each week that they decide not to put me out with the trash I feel blessed. They take it in stride when my office looks like a site in need of FEMA assistance and are kind enough not to rearrange things, disrupting the experiment in chaos theory that is my desk. They know when to offer encouragement and when to offer cheesesteak. Noah, Josh, and Safura, you three mean the world to me, and without your support, I couldn't have done this. I love you all very much.

Reviewers

Even more smart people—as always, a talented team of microbiologists had my back. The information they provided about content, procedures, what I got right, and what I could improve upon made this a better book than I could have ever written on my own. Your feedback was very valuable.

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And that's how you write a book.

To the Student

As an introductory student in microbiology, you may find that the reasons behind a particular exercise appear overly complex. Such is the nature of science, but the reasons should, at the very least, be apparent. The first step in closing the chasm between the scientific and the everyday is to understand, always, how each step relates to the overall objective. It is just as important to understand **why** you are doing something as it is to understand **what** it is you are doing. If you can master both the **why** and the **what,** then your success in microbiology will be assured.

This book was written with you in mind, with each feature designed to support something else. Put another way, the introductory material helps to explain the case study, the photographs and diagrams are used to clarify procedures, the glossary contains definitions of microbiological terms, and websites are provided if you would like further information on a topic. When you are using this book, please, **use** this book. If the meaning of a sentence is unclear, look to the accompanying figure; if a word is a mystery, use the glossary; if space is provided for a detailed drawing, give it your best shot—it will all be important soon. A well-used book becomes weathered as knowledge moves from the book to the reader, and a lab book is no different in this regard. Dog-eared pages, drawings, notes, and circled definitions are all part of learning, and the physical process of making the book yours parallels the intellectual process of making the information yours. This is as true with microbiology as it is with any other interest, job, or hobby. Take the steps to own the book, and you'll own the information within. Good luck. Work hard and have fun.

About the Author

©Noah Chess

Barry Chess has taught microbiology at Pasadena City College for more than 20 years. Prior to that, while studying at the California State University and the University of California, he conducted research into the expression of genes involved in the development of muscle and bone.

At Pasadena City College, beyond his usual presence in the microbiology laboratory and lecture hall, Barry has taught majors and nonmajors biology, developed a course in human genetics, helped to found a biotechnology program on campus, and regularly supervises students completing independent research projects in the life sciences. Over the past several years, his interests have focused on innovative methods of teaching that lead to greater student success. He has written and reviewed cases for the National Center for Case Study Teaching in Science and contributed to the book *Science Stories You Can Count On: 51 Case Studies with Quantitative Reasoning in Biology*. Barry has presented papers and talks on the effective use of case studies in the classroom, the use of digital tools to enhance learning, and for several years served as a scientific advisor for the American Film Institute. In addition to *Laboratory Applications in Microbiology,* Barry is coauthor of the lecture text *Foundations in Microbiology,* now in its tenth edition. He is a member of the American Association for the Advancement of Science and the American Society for Microbiology. Barry was profiled in the book *What Scientists Actually Do,* where he was illustrated as a young girl with pigtails, about to stick a fork into an electrical outlet.

LABORATORY SAFETY GUIDELINES

The microbiology lab presents a number of safety challenges, many of which are unique to this field of study. The following guidelines should be followed to help ensure your safety in the microbiology laboratory.

1. Be realistic if you feel you shouldn't be in the lab because of health concerns. Conditions that may leave you vulnerable to infection such as a short-term illness, being immunocompromised, taking immunosuppressant drugs, or being pregnant should be candidly discussed with your instructor.
2. Dress appropriately for the lab. No open toed shoes or sandals. Clothing with baggy sleeves that could catch fire or hinder your movements should be avoided.
3. Know where the safety equipment is in the lab. Note the location of the eye wash, safety shower, fire extinguisher, and first aid kit. Take a moment to learn their operation.
4. Always wear a lab coat while in the lab. Even if you are not working yourself, another person in the lab could have an accident. This garment should only be used during lab and should remain in the laboratory.
5. Wash your hands prior to beginning lab and just before leaving as well. Also wash when removing gloves and if you feel you may have contaminated yourself.
6. Tie back long hair. It is both a source of contamination and a fire hazard.
7. Nothing should go into your mouth during lab. Do not eat or drink in the lab, even if no work is being done at the time.
8. Do not apply makeup and never handle contact lenses in the lab.
9. Always wear gloves when handling blood or blood products.
10. Wash with an antiseptic if your skin is exposed to microorganisms as a result of a spill.
11. Dispose of broken glass, needles, lancets, wooden applicators and any other object that could penetrate the skin, in a hard sided sharps container. Do not overfill the container and never, ever force objects into the container.
12. Make use of fume hoods when undertaking procedures where noxious chemicals may be released during heating.
13. In the event of a spill, notify your instructor immediately.

CASE STUDY EXERCISE 1

Safety Considerations in the Microbiology Laboratory

STUDENT LEARNING OUTCOMES

After completing this exercise, you should be able to:

1. Demonstrate proper primary and secondary containment procedures.
2. Explain the procedures involved in dealing with a laboratory emergency.

INTRODUCTION

The microbiology laboratory presents a number of unique challenges. Not only are the normal hazards of a laboratory environment (flames, caustic chemicals, and glassware) present, so too are infectious organisms. In fact, the microbiology lab is devoted to growing and studying the very organisms that may cause us such harm! Laboratory workers are by no means immune to infection in the laboratory, and it is not an exaggeration to say that proper safety procedures can be a matter of life and death.

Safe laboratory procedures revolve around containment of microorganisms. **Primary containment** concerns the protection of personnel and the laboratory environment from exposure to infectious microbes. Proper microbiological techniques, such as the safe transport and disposal of cultures, along with the correct use of personal safety equipment (e.g., gloves and safety glasses) go a long way toward accomplishing the goal of personal containment. **Secondary containment** deals with protecting the outside environment from exposure to infectious organisms and depends principally on the design of the laboratory and the availability of equipment. Most laboratory workers can do little to influence the physical aspects of the laboratory other than not disabling safety features, such as keeping open a door that should remain closed, turning off an exhaust fan, or removing a fire extinguisher.

> #1 Rule: No eating or drinking in the lab! After all, how would you feel if I practiced microbiology in your kitchen?

The type of organisms dealt with in the laboratory will dictate the safety precautions used. Working with deadly viruses obviously requires a greater degree of vigilance than working with bacteria that are nonpathogenic. To clarify exactly what techniques and equipment should be used, microorganisms are classified into one of four biosafety levels (BSL-1 through BSL-4) based on their ease of transmission and pathogenicity. Each level has a set of minimum standards with regard to laboratory practices, equipment, and facilities. At one end, BSL-1 organisms generally do not cause disease in a healthy person and require very few specialized techniques. BSL-4 organisms, in contrast, are easily transmitted and cause life-threatening diseases. BSL-4 laboratories are the stuff of science fiction, with full-body spacesuits, respirators, and showers upon exiting the laboratory. Table 1.1 summarizes the recommended biosafety levels for selected infectious agents.

The vast majority of introductory microbiology laboratories are designed to handle BSL-1 and BSL-2 rated organisms, and the rules that apply in these laboratories have a very common sense feel about them. Your instructor may modify these rules based on college, municipal, or state regulations as well as the organisms you are likely to work with as part of your course. Adhering to these guidelines will help to ensure your safety in the microbiology lab.

Prior to the Lab

- Dress appropriately for the lab. No open-toed shoes or sandals. Clothing with baggy sleeves that could catch fire or hinder your movements should be avoided.
- Know where the safety equipment is in the lab. Note the location of the eye wash, safety shower, fire extinguisher, and first aid kit. Take a moment to learn their operation; remember, if you need to use the eyewash, you very well may not be able to see at the time.

During the Lab

- Always wear a lab coat while in the lab. Although you may not be working yourself, another person in the lab could have an accident. This garment should only be used during lab and should remain in the lab. Even discounting potential biohazards, a lab coat will protect your clothing. There is a reason many of the chemicals you will be working with are called stains.
- Wash your hands prior to beginning the lab and just before leaving as well. Also wash after removing gloves or if you feel you may have contaminated yourself. If your laboratory sink has a hands-free method of activating the flow of water (such as foot pedals), use it.
- Tie back long hair, it is both a source of contamination and a fire hazard.
- Disinfect your benchtop with amphyl, Lysol, or 10% bleach prior to beginning work and just before leaving the laboratory. If time permits, allow the disinfectant to evaporate rather than wiping the surface of your bench dry.
- Keep clutter on your bench to a minimum. Store book bags, purses, and other unneeded items where they will not consume

TABLE 1.1 Summary of biosafety levels for selected infectious agents

BSL	Health risk	Practices	Primary barriers	Secondary barriers	Organisms (Selected examples)
1	Not known to cause disease in healthy individuals.	Open bench microbiology.	None required.	Open benchtops and sinks.	*Micrococcus luteus* and *Bacillus megaterium.*
2	Can cause disease in healthy people, but organisms are easily contained.	Limited lab access and biohazard warning signs.	Gloves, lab coat, eye protection, and/or face shield as needed.	BSL-1 plus: • Access to autoclave.	*Escherichia coli* and *Staphylococcus aureus*, along with most other human pathogens.
3	Can cause severe disease, especially when inhaled.	BSL-2 plus: • Controlled access to lab. • No unsterilized material can leave the lab. • Decontamination of clothes prior to laundering.	BSL-2 plus: • Biosafety cabinets used for all manipulations.	BSL-2 plus: • Access to self-closing double doors. • Negative pressure (air flows into lab from outside). • Exhausted air not recirculated.	*Mycobacterium tuberculosis,* HIV, and *Yersinia pestis.*
4	Highly virulent microbes posing extreme risk to humans, especially when inhaled.	BSL-3 plus: • Clothing must be changed before entering the lab, and personnel must shower upon exiting the lab. • All material is decontaminated prior to leaving the facility.	BSL-3 plus: • All procedures are conducted in complete isolation biosafety cabinets (BSCs) or in class I or II BSCs along with full-body, positive-pressure suits with supplied air.	BSL-3 plus: • Isolated building or lab. • Isolated laboratory systems (air supply and exhaust, vacuum, and decontamination).	Lassa fever virus, Ebola virus, and Marburg virus.

precious bench space and where they will be less likely to be contaminated by an inadvertent spill.

- Put your cell phone away! Cell phones held to your mouth substantially increase the risk of accidental infection and can easily transport bacteria out of the laboratory. Switching between lab work and texting is an equally bad idea.

Your cell phone has no place in the lab. Don't rely on your phone as a calculator or timer.

- Nothing should go into your mouth while you are in the laboratory. Do not eat or drink in the lab, even if no work is being done at the time.
- Skin and eyes represent a common portal of entry for pathogens. Do not apply makeup, and never handle contact lenses in the lab.
- Organize your workplace before beginning (Figure 1.1). Store culture tubes upright in a rack, never on their side. Caps on tubes are generally not tight fitting, and liquid will leak out (even from a solid culture), leading to contamination.
- The open flame produced by a Bunsen burner presents an obvious danger in the laboratory. Burners should be set up away from overhead equipment or shelving, and the immediate area should be free of combustible materials such as notes or books. Prior to lighting the burner, quickly inspect the hose for holes, cracks, or leaks, and be sure it fits securely on both the gas valve and the burner.

Figure 1.1 Personal protection in the laboratory includes the use of a lab coat, gloves, and eye protection. Also note that long hair is tied back and the work area is free of clutter. ©nandyphotos/Getty Images

Disposal of Contaminated Materials

Most material in the microbiology laboratory must be decontaminated prior to being disposed of or reused, and this is most often accomplished using an autoclave (Figure 1.2), which uses steam under high pressure to kill even the most resistant organisms. After decontamination, culture tubes, glass pipettes, and the like are washed and reused. Plastic Petri dishes melt during the decontamination process and are discarded after autoclaving along with single-use items such as tongue depressors, needles, and swabs. In general, disposal of lab materials depends on whether or not it will be reused. In any event, the contents of plates or tubes should never be touched by hand.

- Dispose of plastic Petri dishes, swabs, disposable gloves, and similar nonreusable items in a biohazard bag (Figure 1.3a). Petri dishes should be taped closed, but there is no need to remove labels or tape from items.
- Reusable supplies such as culture tubes and glass pipettes should have all labels removed before being placed in a rack or container designated for autoclaving.
- Used microscope slides should be placed in a container for autoclaving or soaked in a disinfectant solution for a minimum of 30 minutes before being discarded.

Safety Considerations

- Be realistic if you feel you shouldn’t be in lab because of health concerns. Conditions that may leave you vulnerable to infection such as a short-term illness, being immunocompromised, taking immunosuppressant drugs, or being pregnant should be candidly discussed with your instructor.
- Always wear gloves when handling blood or blood products. Bloodborne pathogens have special procedures associated with them, and work of this type should only be done with the explicit knowledge of your instructor.
- Wash with an antiseptic if your skin is exposed to microorganisms as a result of a spill.
- Dispose of broken glass, needles, lancets, wooden applicators, and any other object that could penetrate the skin, in a hard-sided sharps container (see Figure 1.3b). Do not overfill the container, and never, ever force objects into the container.
- Biosafety cabinets are sometimes used to work more safely with BSL-2 organisms (figure 1.4). BSL-3 and BSL-4 organisms have more stringent requirements.

(a) (b)

Figure 1.3 All disposable, potentially infectious waste should be placed in a biohazard container, with needles, slides, tongue depressors, and anything else that could penetrate a plastic bag restricted to disposal in a hard-sided receptacle. The international biohazard symbol on these containers not only marks the contents for autoclaving prior to disposal but also cautions anyone in the room as to the possibly hazardous nature of the items inside the container. (a) ©McGraw-Hill Education/Tim Fuller, photographer; (b) ©McGraw-Hill Education/Sandra Mesrine, photographer

Figure 1.2 Autoclaves use steam under pressure to sterilize biohazardous materials prior to disposal. ©Barry Chess

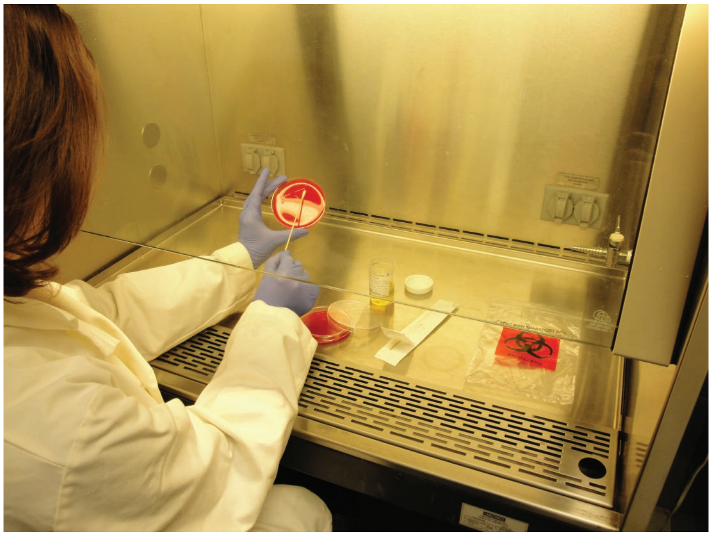

Figure 1.4 A biosafety cabinet can be used to enhance protection when working with potential pathogens. Note that the glass shield is lowered as far as is practical and that clutter within the cabinet is kept to a minimum. The vents at the bottom of the cabinet are connected to a powerful vacuum that creates a curtain of air, further helping to protect the user from organisms being manipulated within the cabinet. ©McGraw-Hill Education/James Redfearn, photographer

- In the event of a spill, notify your instructor immediately. Broken glass and bacterial cultures are a hazardous combination. With the instructor's approval, cover the spill with paper towels and saturate the towels with disinfectant. After 10 minutes, carefully wipe up the spill and discard the paper towels in the biohazard container for autoclaving. Discard the broken glass in the sharps container.

PRE-LAB QUESTIONS

Remember, Understand, Apply

1. A mechanism that automatically closes a door leading into a laboratory would be considered an example of
   - **a.** primary containment.
   - **b.** secondary containment.
   - **c.** tertiary containment.
   - **d.** quaternary containment.
2. The most dangerous microbiological pathogens are classified as
   - **a.** BSL-1.
   - **b.** BSL-2.
   - **c.** BSL-3.
   - **d.** BSL-4.
3. The laboratory benchtop should be cleaned at least ______ per laboratory period.
   - **a.** once
   - **b.** twice
   - **c.** three times
   - **d.** four times
4. You should refrain from eating and drinking in the lab until both you and your immediate neighbors have finished working.
   - **a.** true
   - **b.** false
5. Liquid cultures should be disposed of by
   - **a.** pouring the contents of the tube into the sink and then washing the tube.
   - **b.** discarding the tube into the nearest trash can.
   - **c.** removing any labels from the outside of the tube and placing the tube in a rack for disinfecting.
   - **d.** pouring disinfectant into the tube to kill the culture, then washing the tube.
6. In the laboratory, cell phones
   - **a.** should be nearby in case emergency help is required.
   - **b.** should be used only for texting, calculating, or the timing of reactions, so the phone is not brought close to your mouth.
   - **c.** should be kept away from the bench and not used at all.
   - **d.** should be disinfected at the end of the laboratory period.
7. Personal safety equipment and practices in a BSL-1 or BSL-2 laboratory would generally include all of the following *except*
   - **a.** refraining from eating and drinking in the laboratory.
   - **b.** tying back long hair.
   - **c.** wearing a mask over your nose and mouth.
   - **d.** wearing shoes that cover the entire foot.
8. A wooden applicator stick that had been used to sample bacteria should be disposed of
   - **a.** in a wastepaper basket.
   - **b.** in a biohazard bag.
   - **c.** in a hard-sided biohazard container.
   - **d.** in a container filled with disinfectant.

PROCEDURE

1. Identify the location of each of the following pieces of safety equipment in your laboratory.

   Lab coat storage

   Fire extinguisher

   Disinfectant

   First aid kit

   Safety shower

   Eyewash station

   Biohazardous waste disposal

   Sharps container

REVIEW QUESTIONS

Analyze, Evaluate, Create

1. Classify each of the following agents as BSL class 1, 2, 3, or 4 based on its description.

Description	Severity of disease	Mode of transmission	BSL level
Ebola virus	Lethal in 50%–90% of cases. No effective treatment.	Direct contact or body fluids.	
Mycobacterium tuberculosis	Severe, but treatable respiratory disease.	Respiratory droplets.	
Bacillus subtilis	Does not cause disease in immunocompetent persons.	Not easily transmitted.	
Clostridium tetani	Can be lethal in nonprotected individuals. Vaccine provides protection.	Anaerobic sites (deep puncture wounds).	

2. Explain, as specifically as possible, how each of the following helps to enhance safety in the microbiology lab. Explain whether each is necessary in all instances.

Negative air flow (i.e., air flows into the laboratory rather than out)

Gloves, safety glasses, and lab coat

Foot pedal activation of sinks

Prohibitions on eating and drinking in the lab

3. How would you properly dispose of each of the following items?

A Petri dish containing a fungal culture

A glass culture tube containing a bacterial culture

A spill containing broken glass and a bacterial culture

CASE STUDIES

The following cases illustrate the very real dangers of working in a microbiology laboratory. Study the details of each case and use your knowledge of safe laboratory practices to answer the questions which follow.

Community College Acquired Infection with *Salmonella* Typhimurium—United States, 2017.

In December 2011 three persons suffering from *Salmonella* Typhimurium infection were identified by the New Mexico Department of Health. *Salmonella* infections of this type are usually marked by diarrhea, fever, and cramps lasting four to seven days, but more severe cases require hospitalization, and death is a distinct possibility if the bacterium enters the bloodstream. The New Mexico Department of Health took a special interest in these three persons because one was a student and two others were children of students in microbiology classes held at two different community college campuses. A complete investigation by the Centers for Disease Control (CDC) eventually identified 109 patients across a 38-state area sickened by the same bacterium.

Analysis by the CDC found that the greatest risk factor for contracting this strain of *Salmonella* was not eating contaminated hamburger, alfalfa sprouts, or cantaloupe—all of which had been linked to other outbreaks—but rather being recently exposed to a microbiology laboratory. Inadequate adherence to safety procedures had not only sickened many students and employees, but the infectious bacterium was taken home on pens and pencils, cell phones, and backpacks, where family members of the students were then infected. The Centers for Disease Control examined safety practices in a number of labs and found that students in labs where illnesses occurred were less likely to have biosafety training than students in labs where no illnesses occurred. Because many people involved in the outbreak were college students, the median age of those infected was 21 years. In all, 13 hospitalizations and one death were attributed to this completely preventable incident.

Despite a renewed emphasis on laboratory safety, especially in teaching labs, a second outbreak of the same bacterium was seen in 2014. Forty-one people across 13 states were infected, with 36% requiring hospitalization. The median age of those infected was 20 years, and 86% were enrolled in a biology or microbiology course. When surveyed, many ill persons reported engaging in laboratory behaviors that would increase their risk of infection, including failing to wear a lab coat, inadequate handwashing, and using the same writing utensils and notebooks outside of the laboratory.

If that weren't enough, in 2017, a third multistate outbreak of *Salmonella* Typhimurium infections took place. Once again linked to college and university teaching laboratories, the 2017 outbreak affected 24 persons in 16 states, with 6 requiring hospitalization. Interviews with ill persons revealed many of the same lapses in safety seen in the 2011 and 2014 outbreaks.

Scientist Dies of Laboratory Acquired Plague, Chicago, 2009

Dr. Malcolm Casadaban worked at the University of Chicago studying *Yersinia pestis,* the bacterium responsible for bubonic plague. Spread by fleas, plague has been feared for centuries. Epidemics in Europe and Asia were thought to have killed more than 100 million people in the 1300s, shaping migrations patterns and leading to attacks on Jews, friars, foreigners, lepers, and other groups thought responsible for outbreaks. Known as the Black Death because of the necrosis (tissue death) caused by the bacterium, plague continues to cause small numbers of deaths around the world. Plague is a zoonotic disease, meaning that it is most commonly encountered in animals but can be spread to humans. Forest rangers, campers, veterinarians, or anyone who interacts with animals is at increased risk of infection.

Bubonic plague is often referred to as the Black Death because of the necrosis caused by infection with *Yersinia pestis*. Source: CDC /Christina Nelson, MD, MPH

Casadaban was working with *Yersinia pestis* in an attempt to develop an effective vaccine against the plague. The strain with which he was working had been deliberately weakened so that it could not absorb enough iron to make functional enzymes. Even when injected into mice, the bacterium was incapable of causing death.

In mid-September, Casadaban visited his doctor, complaining of "flu-like" symptoms. He was told he most likely had contracted a virus and was directed to rest. Three days later he returned to the doctor, very sick, and died 13 hours later. An autopsy revealed the supposedly innocuous strain of *Yersinia pestis* in his system, but Casadaban's death remained a mystery. How could such a weakened strain of *Yersinia pestis* cause death? Analysis of the doctor's blood finally solved the puzzle. Unbeknownst to him, Dr. Casadaban suffered from hemochromatosis, a genetic disorder in which people accumulate high levels of iron in their blood. This excess of iron allowed the usually iron-starved *Yersinia pestis* to assume its original virulence. Further investigation revealed that he occasionally went without gloves in the laboratory, thinking the bacterium was entirely incapable of causing infection. Dr. Ken Alexander, an infectious disease specialist and colleague of Dr. Casadaban, said that the dead researcher would have had one comment for those scientists investigating his death, "Listen guys, I'm trying to teach you something—and you better damn well learn it."

Laboratory Researcher Dies after Suffering Burns—Los Angeles, California, 2009–2018

Sheri Sangji, a 23-year-old research assistant at UCLA, was transferring a small quantity of t-butyl lithium from one container to another when a plastic syringe came apart in her hands, splashing her with a chemical compound that ignites instantly when exposed to air. She received second and third degree burns over 43% of her body and died 18 days later.

Sangji was a recent college graduate who had only been working in the lab at UCLA for a few months when the incident occurred. An investigation by the California Division of Occupational Safety and Health concluded that she had not been properly trained for the procedure she was undertaking and did not know what to do in the event she caught fire. Sangji was not wearing a protective lab coat and was dressed in a nylon sweater described as "solid gasoline" by a lab safety expert. A previous inspection of the lab by UCLA safety personnel turned up several safety violations, which had not been corrected at the time of the accident. UCLA, as part of a plea agreement with the Los Angeles County district attorney's office, agreed to accept responsibility for the lab's operating conditions at the time of the fire, to follow comprehensive safety measures, and to endow a $500,000 scholarship in Sangji's name.

Patrick Harran, the professor in whose lab the accident occurred, was arrested on felony charges, the first instance of a criminal case arising from an academic laboratory accident. As part of a plea agreement, he promised to develop and teach a chemistry course for inner-city students, perform 800 hours of community service in the UCLA hospital system, and pay $10,000 to the Grossman Burn Center, where Sangji died. If the professor complies with the details of the plea agreement, the charges against him will be dismissed.

Throughout the trial, UCLA and Dr. Harran portrayed Sangji as an experienced chemist who had been properly trained and chose not to wear protective gear while she worked in the lab, though others had very different opinions. Said Neal Langerman, the previously mentioned lab safety expert, concluded, "Poor training, poor technique, lack of supervision, and improper method . . . She died, didn't she? It speaks for itself."

CASE STUDY ANALYSIS

1. For each of the cases seen here, postulate where the breakdown in laboratory safety occurred and suggest how it could be corrected.

Salmonella infection

Plague infection

Laboratory fire

2. The incident of plague infection seen in this case study is interesting primarily because a medical condition of the researcher put him alone at risk of infection. Provide two or three examples of common medical or biological conditions that could make working in the microbiology lab especially hazardous. Be sure to justify your answers.

REFERENCES

CDC. 2012. Investigation Update: Human *Salmonella* Typhimurium Infections Associated with Exposure to Clinical and Teaching Microbiology Laboratories. http://www.cdc.gov/salmonella/typhimurium-laboratory/011712/index.html

Christensen, Kim. June 20, 2014. UCLA professor strikes deal in lab fire case, avoids prison. *Los Angeles Times*.

MacKenzie, Debora. March 1, 2011. Plague scientist dies of . . . the plague. *New Scientist.*

U.S. Department of Health and Human Services. 2009. *Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th ed.* www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

CASE STUDY EXERCISE 2

Microscopy and Measurement of Microscopic Specimens

STUDENT LEARNING OUTCOMES

After completing this exercise, you should be able to:

1. Properly use and care for a brightfield microscope.
2. Explain the theories underlying optical microscopy.

INTRODUCTION

Whether we are discussing our skin, plaque on our teeth, a glass of milk, or a swimming pool, nearly everything in our day-to-day existence is loaded with microorganisms. Being able to view these creatures is essential to an understanding of microbiology. It should come as no surprise that the proper use of a microscope is a central skill in microbiology, and the placement of this laboratory exercise near the beginning of this book is no accident.

LIGHT MICROSCOPE

Components of the Light Microscope

The instrument most commonly seen in microbiology labs is the brightfield microscope, so named because when objects are examined, they appear as dark objects in a bright visual field. Although your microscope may differ slightly in appearance from the one seen in this exercise, in theory and practice, the same rules will apply. The components and functions of each of the parts found in a typical brightfield microscope (Figure 2.1) are outlined here.

Framework The frame of a microscope consists of the arm and base. Keep in mind when holding the microscope that these are the only two parts built to support its weight.

Lamp A light source is located in the base of a microscope. A rotating wheel or knob can be used to adjust the voltage received by the lamp, which in turn adjusts the intensity of the light. Many microscopes will also have a blue filter that can be placed over the light source to reduce the intensity of the light and increase the resolution of the microscope.

Diaphragm The diaphragm is an adjustable disc with a hole in the center. The size of the hole can be varied to allow more or less light to pass to the slide by use of a dial or lever.

Condenser The first of three lens systems found on all microscopes, the condenser is located beneath the stage and is usually contained in the same housing as the diaphragm. The condenser collects and focuses light on the specimen being studied. Although the condenser can be raised or lowered, best results in the microbiology lab will be obtained when the condenser is kept at its highest point, just below the level of the stage.

Stage The platform that supports the slide is known as the stage. Most microscopes have a clamping device, the mechanical stage, that allows the slide to be held and moved with greater precision.

Objective Lens Three or four objective lenses, more commonly referred to as objectives, are found just above the stage. The objectives are attached to a revolving **nosepiece** that allows the lenses to be rotated into position. Most microscopes will have three objectives with magnifications of 10x, 45x, and 100x, designated as **low power, high dry**, and **oil immersion**, respectively. Occasionally a 4x **scanning** objective will also be present, but it tends to be of little use in most microbiology labs.

Ocular Lens The third set of lenses, those closest to your eyes, are the ocular lenses. In most instances, these lenses have a magnification of 10x. **Binocular** microscopes have two sets of lenses while **monocular** microscopes have only a single ocular. Binocular microscopes will also have a means of adjusting the distance between the oculars. One ocular may also have a small ring that allows the focus of that ocular to be adjusted independently of the rest of the microscope.

Focus Adjustment Two concentric focusing knobs are located on each side of the microscope. The large outer knob is the **coarse focus adjustment**, while the smaller, inner knob is the **fine focus adjustment**.

Care of the Light Microscope

Microscopes are delicate instruments, and care must be taken in their use. Some general rules related to microscope care include:

Transport The microscope should always be held with two hands. One hand should grasp the microscope around the arm while the second supports the instrument from the bottom. The biggest danger in carrying a microscope with one hand is not that it will be dropped but rather that the scope will collide with the corner of a lab bench or other piece of furniture. Once at your bench, place the scope gently on the table.