Johnson | Case

Laboratory Experiments in microbiology

Eleventh Edition

LABORATORY EXPERIMENTS IN MICROBIOLOGY

ELEVENTH EDITION

TED R. JOHNSON

St. Olaf College

CHRISTINE L. CASE

Skyline College

PEARSON

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Cover Photo Credits: Background image TEK Image/Science Photo Library/Getty Images. *Serratia marcescens* growing on nutrient agar courtesy of Christine L. Case. *Serratia marcescens* is distinguished by its red pigment. In hospital situations, this bacterium can be found on catheters and in wash basins. *S. marcescens* is an important cause of healthcare-associated infections, especially in neonatal intensive care units and in post-surgical patients. *S. marcescens* may cause pneumonia, wound infections, urinary tract infections, endocarditis, bacteremia, and septicemia.

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Contents

APPENDICES 459

Preface

Now in its Eleventh Edition, *Laboratory Experiments in Microbiology* is valued by instructors and students for its comprehensive coverage of every area of microbiology, its user-friendly laboratory reports, and its clear, straightforward organization. Containing 57 thoroughly class-tested exercises, this manual provides basic microbiology techniques with applications for undergraduate students in diverse areas, including the biological sciences, the allied health sciences, agriculture, environmental science, nutrition, pharmacy, and various preprofessional programs. It is designed to supplement any nonmajors microbiology textbook. Coauthored by Christine L. Case, this manual is an ideal companion to *Microbiology: An Introduction,* Twelfth Edition, by Gerard J. Tortora, Berdell R. Funke, and Christine L. Case.

OUR APPROACH

Exercises have been designed to include the American Society for Microbiology laboratory core curriculum, which is considered essential to teach in every introductory microbiology laboratory, regardless of its emphasis.

This laboratory manual has two primary goals—teaching microbiology techniques and showing students the importance of microbes in our daily lives and their central roles in nature. Most of the exercises are investigative by design and require the students to evaluate their experimental results and draw conclusions. We hope in this way to promote analytical reasoning and provide students with a variety of opportunities to reinforce the technical skills they have learned. We also highlight practical uses of microbiology by including material with direct applications to procedures performed in clinical and commercial laboratories.

With a strong emphasis on laboratory safety, this laboratory manual encourages students not only to learn but also to practice safety techniques so that safety becomes part of their professional behavior. We have included a safety contract that students can hand in to their instructors to indicate that they understand safety requirements. (For specific safety suggestions, see the section titled Specific Hazards in the Laboratory on page 4 and the sections of the Introduction that follow it.) We also alert students with yellow safety boxes at key points in the exercises. These safety boxes are marked with either a biohazard icon ☣, a general safety icon ⚠, or a biosafety level 2 icon BSL-2 indicating appropriate safety techniques.

NEW TO THIS EDITION

For the Eleventh Edition, the overall goal was to make this manual even more navigable and visually effective for students and instructors. The following changes have helped us fulfill that goal.

- **Each exercise has been updated** to reflect the American Society for Microbiology 2012 Guidelines for Biosafety in Teaching Laboratories.
- **Biosafety levels (BSLs)** are noted. Every effort has been made to use biosafety level 1 (BSL-1) organisms. BSL-2 organisms are required in a few exercises to demonstrate specific principles and processes.
- **Each part begins with a case study** to give students a real-world example of the applications of the material they are learning. The solution to the case study requires content from the laboratory exercises in that part.
- **Each exercise includes a Lab and Lecture: Putting it all together** activity available through MasteringMicrobiology.® These activities are designed to help students see how lab and lecture are integrated. Students will use their new information and incorporate their new knowledge into lab.

ORGANIZATION

This manual is divided into 14 parts. The introduction to each part explains the unifying theme for that part. Each Part begins with a Case Study that relates the exercises with clinical applications. Exercises in the first four parts provide sequential development of fundamental techniques. The remaining exercises are as independent as possible to allow instructors to select the most desirable sequence for their course. The exercises are organized as follows.

OBJECTIVES This introductory section defines the specific skills or concepts to be mastered in the exercise. The objectives can easily be used as the basis for assessment.

BACKGROUND This narrative section provides definitions and explanations for each exercise. Students should refer to their text for more detailed explanations of the concepts introduced in the laboratory exercises.

MATERIALS This comprehensive list includes supplies, media, and equipment needed for the exercise.

CULTURES This list identifies the living organisms required for the exercise.

TECHNIQUES REQUIRED This section provides a list of techniques from earlier exercises in the manual needed to complete the current exercise.

PROCEDURE At the core of each exercise, this section provides step-by-step instructions, stated as simply as possible and frequently supplemented with diagrams. Questions are occasionally asked in the Procedure section to remind the student of the rationale for a step.

LABORATORY REPORT Designed to help students learn to collect and present data in a systematic fashion, the laboratory report concludes each exercise. Students are first asked to write the *Purpose* of the exercise so they can relate their laboratory work to their learning. Students are asked to formulate their *Hypothesis* or write their *Expected Results* using information provided in the Background and their own experience. Tables are provided to record *Results.* The questions in *Conclusions* are designed to lead the student from a collection of data or observations to a conclusion. In most instances, the results for each student team will be unique; they can be compared with the information given in the Background and other references but will not be identical to that information. The *Questions* reinforce the conclusions and ask the student to interpret results. The range of questions requires students to think about their results, recall facts, and then use this information to answer the questions. *Critical Thinking* questions are designed to help students use their new knowledge and practice analytical skills. *Clinical Application* questions have been collected from the literature. They are designed to encourage students to synthesize their new information to relate concepts and techniques to clinical applications. This lab manual and the course textbook should provide sufficient background to enable students to answer *Critical Thinking* and *Clinical Application* questions.

PREPARATION GUIDE

The comprehensive *Preparation Guide for Laboratory Experiments in Microbiology,* Eleventh Edition (ISBN 0-134-02449-4) provides all the information the instructor needs to set up and teach a laboratory course with this manual. It includes the following:

- General instructions for setting up the lab
- Information on obtaining and preparing cultures, media, and reagents, and expected results for each of the biochemical tests and cultures used
- A master table showing the techniques and biosafety level required for each exercise
- Cross-references for each exercise to specific pages in Tortora/Funke/Case *Microbiology: An Introduction,* Twelfth Edition.
- For each exercise: helpful suggestions, detailed lists of materials needed, and answers to all the questions in the student manual

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

ACKNOWLEDGMENTS

We are most grateful to the following individuals for their time, talent, and interest in our work. Each person carefully read and edited critical parts of the manuscript.

Chuck Hoover of the University of California, San Francisco, for making us aware of the new techniques used in dental microbiology for Exercise 47.

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We are indebted to St. Olaf College and Skyline College for providing the facilities and resources in which innovative laboratory exercises can be developed. We are grateful for the wonderful students who have inspired us and have made teaching microbiology a joy.

We would like to commend the staff at Pearson Education for their support. In particular, we thank Kelsey Churchman, our Acquisitions Editor; Chriscelle Palaganas and Ashley Williams for their editorial skill and conscientiousness; and Nancy Tabor and Courtney Towson for expertly guiding this manual through the production process.

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Last, but not least, our gratitude goes to Michelle Johnson, who gave her professional insights and was a sustaining presence; and Don Biederman, who provided timely encouragement and support.

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A SPECIAL NOTE TO STUDENTS

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

Ted R. Johnson and Christine L. Case

Introduction

LIFE *would not long remain possible in the* **ABSENCE** *of microbes.* **–LOUIS PASTEUR**

Welcome to microbiology! Microorganisms are all around us, and as Pasteur pointed out over a century ago, they play vital roles in the ecology of life on Earth. In addition, some microorganisms provide important commercial benefits through their use in the production of chemicals (including antibiotics) and certain foods. Microorganisms are also major tools in basic research in the biological sciences. Finally, as we all know, some microorganisms cause disease—in humans, other animals, and plants.

In this course, you will have firsthand experience with a variety of microorganisms. You will learn the techniques required to identify, study, and work with them. Before getting started, you will find it helpful to read through the suggestions on the next few pages.

SUGGESTIONS TO HELP YOU BEGIN

1. Science has a vocabulary all its own. New terms will be introduced in **boldface** throughout this manual. To develop a working vocabulary, make a list of these new terms and their definitions.

2. The microbes used in the exercises in this manual are referred to by their *scientific names.* Common names were never given to microbes because they are not visible to the human eye without a microscope. The word *microbe,* now in common use, was introduced in 1878 by Charles Sedillot. The scientific names will be unfamiliar at first, but do not let that deter you. Practice saying them aloud. Most scientific names are taken from Latin and Greek roots. If you become familiar with these roots, the names will be easier to remember.

3. Microbiology usually provides the first opportunity that undergraduate students have to experiment with *living organisms.* Microbes are relatively easy to grow and lend themselves to experimentation. Because there is variability in any population of living organisms, not all the experiments will "work" as the lab manual says. The following exercise will illustrate what we mean:

Write a description of *Homo sapiens* for a visitor from another planet: ______________________

____

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After you have finished, look around you. Do all your classmates fit the description exactly? Probably not. Moreover, the more detailed you make your description, the less conformity you will observe. During lab, you will make a detailed description of an organism and probably find that this description does not match your reference exactly.

4. Microorganisms must be cultured or grown to complete most of the exercises in this manual. Cultures will be set up during one laboratory period and will be examined for growth in the next laboratory period. Accurate record keeping is therefore essential. Mark the steps in each exercise with a bright color or a bookmark so you can return to complete your Laboratory Report on that exercise. *Accurate records* and *good organization* of laboratory work will enhance your enjoyment and facilitate your learning.

5. *Observing* and *recording* your results carefully are the most important parts of each exercise. Ask yourself the following questions for each experiment:
What did the results indicate?
Are they what I expected? If not, what happened?

6. If you do not master a technique, try it again. In most instances, you will need to use the technique again later in the course.

7. Be sure you can answer the questions that are asked in the Procedure for each exercise. These questions are included to reinforce important points that will ensure a successful experiment.

8. Finally, carefully study the general procedures and safety precautions that follow.

GENERAL PROCEDURES IN MICROBIOLOGY

In many ways, working in a microbiology laboratory is like working in the kitchen. As some very famous chefs have said,

> *Our years of teaching cookery have impressed upon us the fact that all too often a debutant cook will start in enthusiastically on a new dish without ever reading the recipe first. Suddenly an ingredient, or a process, or a time sequence will turn up, and there is astonishment, frustration, and even disaster. We therefore urge you, however much you have cooked, always to read the recipe first, even if the dish is familiar to you. . . . We have not given estimates for the time of preparation, as some people take half an hour to slice three pounds of mushrooms, while others take five minutes.**

1. Read the laboratory exercises *before* coming to class.
2. *Plan* your work so that you complete all experiments during the assigned laboratory period. A good laboratory student, like a good cook, is one who can do more than one procedure at a time—that is, one who is efficient.
3. Use only the *required* amounts of materials, so that everyone can do the experiment.
4. *Label* all of your experiments with your name, date, and lab section.
5. Even though you will do most exercises with another student, you must become familiar with *all* parts of each exercise.
6. Keep *accurate* notes and records of your procedures and results so that you can refer to them for future work and tests. Many experiments are set up during one laboratory period and observed for results in the next laboratory period. Your notes are essential to ensure that you perform all the necessary steps and observations.
7. *Demonstrations* will be included in some of the exercises. Study the demonstrations and learn the content.
8. If you are color-blind, let your instructor know; many techniques require discrimination of colors.
9. Keep your cultures current; discard old experiments.
10. *Clean up* your work area when you are finished. Leave the laboratory clean and organized for the next student. Remember:
    - Return stain and reagent bottles to their original locations.
    - Place slides in the appropriate disinfectant container as instructed.
    - Remove all markings on glassware (such as Petri plates and test tubes) before putting glassware into the marked autoclave trays.
    - Place glass Petri plates agar-side down in marked autoclave containers.
    - Place swabs and pipettes in the appropriate disinfectant jars or biohazard containers.
    - Place disposable plasticware in marked biohazard or autoclave containers.
    - Discard used paper towels.

*J. Child, L. Bertholle, and S. Beck. *Mastering the Art of French Cooking,* Vol. 1. New York: Knopf, 1961.

BIOSAFETY

The most important element for managing microorganisms is strict adherence to standard microbiological practices and techniques, which you will learn during this course. There are four biosafety levels (BSLs) for working with live microorganisms; each BSL consists of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. (See Table 1 on page 3.) Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the microorganisms, and the laboratory function or activity.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no specific facilities other than a sink for handwashing. When standard laboratory practices are not sufficient to control the hazard associated with a particular microorganism, additional measures may be used. Gloves should be worn if skin on hands is broken or if a rash is present.

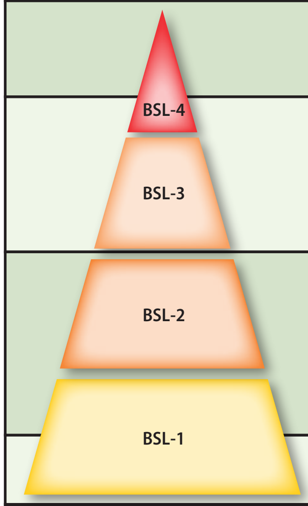
Biosafety Level 2 includes handwashing, and an autoclave for decontaminating laboratory wastes must be available. Precautions must be taken for handling and disposing of contaminated needles or sharp instruments. BSL-2 is appropriate for working with human body fluids. A lab coat should be worn. Gloves should be worn when hands may contact potentially hazardous materials.

Biosafety Level 3 is used in laboratories where work is done with pathogens that can be transmitted by the respiratory route. BSL-3 requires special facilities with self-closing, double doors and sealed windows.

Biosafety Level 4 is applicable for work with pathogens that may be transmitted via aerosols and for which there is no vaccine or treatment. The BSL-4 facility is generally a separate building with specialized ventilation and waste management systems to prevent release of live pathogens to the environment.

Which biosafety level is your lab? ______________

TABLE 1 BIOSAFETY LEVELS

Biosafety level	Practices	Personal Protection (Primary Barriers)	Facilities (Secondary Barriers)
 BSL-4	BSL-3 plus • Separate building	BSL-3 plus full-body air-supplied, positive-pressure personnel suit	BSL-3 plus separate building and decontamination facility
BSL-3	BSL-2 plus • Controlled access • Decontamination of clothing before laundering	BSL-2 plus protective lab clothing; enter and leave lab through clothing-changing and shower rooms	BSL-2 plus self-closing, double-door access
BSL-2	BSL-1 plus • Limited access • Biohazard warning signs • “Sharps” precautions • Safety manual of waste decontamination policies	Lab coat; goggles and gloves, as needed	BSL-1 plus autoclave
BSL-1	Standard microbiological practices	Lab coat; goggles and gloves, as needed	Open benchtop sink; autoclave recommended

Biosafety Practices

The lab exercises in this course involve the use of living organisms. Although the microorganisms we use are not considered to be highly virulent, all microorganisms should be treated as potential pathogens (organisms capable of causing disease).

The following rules must be observed at all times to prevent accidental injury to or infection of yourself and others and to minimize contamination of the lab environment. These guidelines are in agreement with *Guidelines for Biosafety in Teaching Laboratories* (American Society for Microbiology, 2012).

1. Never place books, backpacks, purses, or the like on benchtops. Always place these in the assigned cubicles.
2. Electronic devices should not be brought into the lab. This includes, but is not limited to, iPods, MP3 players, radios, cell phones, and calculators.
3. Clean your work area with disinfectant at the beginning AND end of each lab.
4. Wash your hands with soap and dry with paper towels when entering and leaving the lab.
5. Wear a lab coat at all times while working in the lab to prevent contamination or accidental staining of your clothing.
   - **a.** Closed-toe shoes (no sandals) are to be worn in the lab.
   - **b.** Tie back long hair to prevent exposure to flame and contamination of cultures.
   - **c.** Gloves should be worn when staining microbes and handling hazardous chemicals.
   - **d.** Wear safety goggles when performing a procedure (such as pipetting, spread plates, and so on) that may generate a splash hazard.
6. Do not place anything in your mouth or eyes while in the lab. This includes pencils, food, and fingers. Keep your hands away from your mouth and eyes.
   - **a.** Eating (including gum, cough drops, and candy) and drinking are prohibited in the lab at all times. Do not bring water bottles into the lab.
   - **b.** Do not apply cosmetics in the lab.
   - **c.** Never pipette by mouth. Use a mechanical pipetting device.
7. Do not remove media, equipment, or bacterial cultures from the laboratory. This is absolutely prohibited and unnecessary.
8. Do not place contaminated instruments such as inoculating loops, needles, and pipettes on benchtops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles.
9. Carry cultures in a test-tube rack when moving around the lab and when keeping cultures on benchtops for use.
10. Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate with disinfectant. Notify your instructor that there has been a spill. After 20 minutes, clean up the area and dispose of the towels and broken items as indicated by your instructor.
11. Report accidental cuts or burns to the instructor immediately.
12. At the end of each lab session, place all cultures and materials in the proper disposal area.
13. Persons who are immunocompromised (including those who are pregnant) and students living with or caring for an immunocompromised individual are advised to consult their physician to determine the appropriate level of participation in the lab.

SPECIFIC HAZARDS IN THE LABORATORY

Keep containers of alcohol away from open flames.

Glassware Not Contaminated with Microbial Cultures

1. If you break a glass object, sweep up the pieces with a broom and dustpan. Do not pick up pieces of broken glass with your bare hands.
2. Place broken glass in one of the containers marked for this purpose. The one exception to this rule concerns broken mercury thermometers; consult your instructor if you break a mercury thermometer.

Electrical Equipment

1. The basic rule to follow is this: Electricity and water don't mix. Do not allow water or any water-based solution to come into contact with electrical cords or electrical conductors. Make sure your hands are dry when you handle electrical connectors.
2. If your electrical equipment crackles, snaps, or begins to give off smoke, do not attempt to disconnect it. Call your instructor immediately.

Fire

1. If *gas burns* from a leak in the burner or tubing, turn off the gas.
2. If you have a *smoldering sleeve,* run water on the fabric.
3. If you have a *very small fire,* the best way to put it out is to smother it with a towel or book (not your hand). Smother the fire quickly.
4. If a *larger fire* occurs, such as in a wastebasket or sink, use one of the fire extinguishers in the lab to put it out. Your instructor will demonstrate the use of the fire extinguishers.
5. In case of a *large fire* involving the lab itself, evacuate the room and building according to the following procedure:
   a. Turn off all gas burners, and unplug electrical equipment.
   b. Leave the room and proceed to ______________

      ____
   c. It is imperative that you assemble in front of the building so that your instructor can take roll to determine whether anyone is still inside. Do not wander off.

Accidents and First Aid

1. Report all accidents immediately. Your instructor will administer first aid as required.
2. For spills in or near the eyes, use the eyewash immediately.
3. For large spills on your body, use the safety shower.
4. For heat burns, chill the affected part with ice as soon as possible. Contact your instructor.
5. Place a bandage on any cut or abrasion.

Earthquake

Turn off your gas jet and get under your lab desk during an earthquake. Your instructor will give any necessary evacuation instructions.

ORIENTATION WALKABOUT

Locate the following items in the lab:

Broom and dustpan	Instructor's desk
Eyewash	Reference books
Fire blanket	Safety shower
Fire extinguisher	To Be Autoclaved area
First-aid cabinet	Biohazard containers
Fume hood	

SPECIAL PRACTICES

Potential pathogens used in the exercises in this manual present a minimal hazard and require ordinary aseptic handling conditions (Biosafety Level 2). They are marked throughout this manual with the **BSL-2** icon. No special competence or containment is required. These organisms are the following:

- *Enterococcus faecalis*
- *Proteus* species
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Str. pyogenes*

BSL-1 labs will not be using the BSL-2 microbes but will observe demonstration cultures using BSL-2 organisms.

Treat all microorganisms subcultured from the environment as potential BSL-2 organisms, and subculture them only if you are in a BSL-2 laboratory.

LABORATORY FACILITIES

1. Interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned.
2. Benchtops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Windows in the laboratory are closed and sealed.
4. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.
5. Keep laboratory doors closed when experiments are in progress.
6. The instructor controls access to the laboratory and allows access only to people whose presence is required for program or support purposes.
7. Place contaminated materials that are to be decontaminated at a site away from the laboratory into a durable, leakproof container that is closed before being removed from the laboratory.
8. An insect- and rodent-control program is in effect.

Student Safety Contract

During your microbiology course, you will learn how to safely handle fluids containing microorganisms. Through practice you will be able to perform experiments in such a way that bacteria, fungi, and viruses remain in the desired containers, uncontaminated by microbes in the environment. These techniques, called **aseptic techniques,** will be a vital part of your work if you are going into health care or biotechnology.

1. Do not eat, drink, smoke, store food, or apply cosmetics in the laboratory.
2. Wear closed-toe shoes at all times in the laboratory.
3. Tie back long hair.
4. Disinfect work surfaces at the beginning and end of every lab period and after every spill. The disinfectant used in this laboratory is ________________.
5. Wash your hands before and after every laboratory period. Because bar soaps may become contaminated, use liquid or powdered soaps.
6. Use mechanical pipetting devices; do not use mouth pipetting.
7. Wear safety goggles while pipetting.
8. Wash your hands immediately and thoroughly with soap and water if they become contaminated with microorganisms.
9. Cover spilled microbial cultures with paper towels, and saturate the towels with disinfectant. Leave covered for 20 minutes, and then clean up the spill and dispose of the towels.
10. Do not touch broken glassware with your hands; use a broom and dustpan. Place broken glassware *contaminated* with microbial cultures or body fluids in the To Be Autoclaved container. (See page 4 for what to do with broken glassware that is not contaminated.)
11. Place glassware and slides contaminated with blood, urine, and other body fluids in disinfectant.
12. To avoid transmitting disease, work only with your own body fluids and wastes in exercises that require saliva, urine, blood, or feces. The Centers for Disease Control and Prevention (CDC) states that "epidemiologic evidence has implicated only blood, semen, vaginal secretions, and breast milk in transmission of HIV" (*Biosafety in Microbiological and Biomedical Laboratories,* www.cdc.gov).
13. Do not perform unauthorized experiments.
14. Do not use equipment without instruction.
15. Do not engage in horseplay in the laboratory.
16. If you got this far in the instructions, you'll probably do well in lab. Enjoy lab, and make a new friend.

Procedures marked with this biohazard icon should be performed carefully to minimize the risk of transmitting disease.

Procedures marked with this safety icon should be performed carefully to minimize risk of exposure to chemicals or fire.

I have read the above laboratory safety rules and agree to abide by them when in the laboratory.

Name: ______________________________ Date: ________________

PART

1

Microscopy

EXERCISES

Antoni van Leeuwenhoek is the first person known to have observed living microbes in suspension. Unfortunately, he was very protective of his homemade microscopes and left no description of how to make them (see the photograph on page 8). During his lifetime he kept "for himself alone" his microscopes and his method of observing "animalcules." Directions for making a replica of van Leeuwenhoek's microscope can be found in *American Biology Teacher.** Fortunately, you will not have to make your own microscope.

The microscope is a very important tool for a microbiologist. Microscopes and microscopy (microscope technique) are introduced in Exercises 1 and 2, which are designed to help you become familiar with the compound light microscope and proficient in using it. This knowledge will be valuable in later exercises.

Beginning students frequently become impatient with the microscope and forgo this opportunity to practice and develop their observation skills. Simple observation is a critical part of any science. Making discoveries by observation requires *curiosity* and *patience.* We cannot provide procedures for observation, but we can offer this suggestion: Make *careful sketches* to enhance effective observation. You need not be an artist to draw what you see. In your drawings, pay special attention to

1. *Size relationships.* For example, how big are bacteria relative to protozoa?
2. *Spatial relationships.* For example, where is one bacterium in relation to the others? Are they all together in chains?
3. *Behavior.* For example, are individual cells moving, or are they all flowing in the liquid medium?
4. *Sequence of events.* For example, were cells active when you first observed them?

Looking at objects through a microscope is not easy at first, but with a little practice, you, like Antoni van Leeuwenhoek, will make discoveries in the microcosms of peppercorn infusions and raindrops. In 1684, van Leeuwenhoek wrote the following:

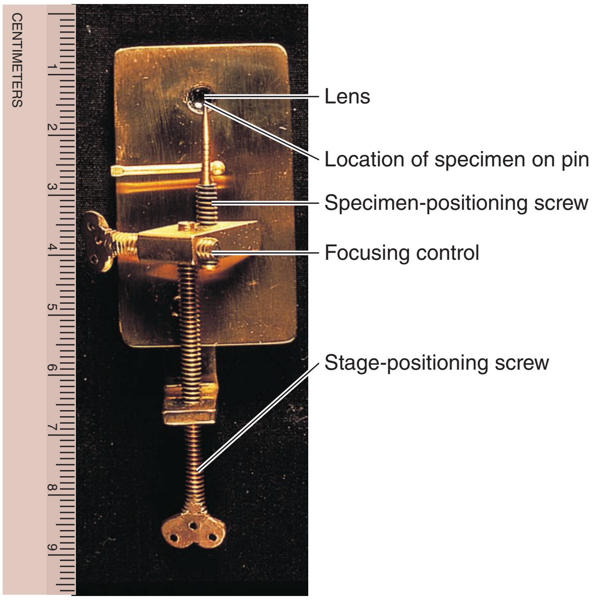
> *Tho my teeth are kept usually very clean, nevertheless when I view them in a Magnifying Glass, I find growing between them a little white matter as thick as wetted flour: In this substance tho I do not perceive any motion, I judged there might probably be living creatures.*

---

*W. G. Walter and H. Via. "Making a Leeuwenhoek Microscope Replica." *American Biology Teacher* 30(6): 537–539, 1968.

*I therefore took some of this flour and mixed it either with pure rain water wherein were no Animals; or else with some of my Spittle (having no air bubbles to cause a motion in it) and then to my great surprise perceived that the aforesaid matter contained very many small living Animals, which moved themselves very extravagantly. Their motion was strong and nimble, and they darted themselves thro the water or spittle, as a Jack or Pike does thro the water.**

*Quoted in E. B. Fred. "Antoni van Leeuwenhoek." *Journal of Bacteriology* 25: 1, 1933.



A replica of the simple microscope made by Antoni van Leeuwenhoek to observe living organisms too small to be seen with the naked eye. The specimen was placed on the tip of the adjustable point and viewed from the other side through the tiny round lens. The highest magnification with his lenses was about 300×.

CASE STUDY: Too Many Slides

Your first microbiology field trip is to a large university hospital lab. Urinary tract infections are quite common, so it is not surprising that urine specimens make up a large proportion of the samples submitted for routine laboratory diagnosis. Nevertheless, you are surprised to learn that the lab technicians may examine 200 microscope slides of urine every day. You are given the opportunity to look at some of the slides and are asked to describe any microorganisms that you see.

Use the following choices to indicate which type of microorganism the one described in each question is most likely to be.

Questions

- **a.** Alga
- **b.** Bacterium
- **c.** Fungus
- **d.** Protozoan

1. In a wet mount of urine, you observe flagellated, nucleated cells. Which type of microorganism is most likely?
2. In a fixed, stained slide, you don't see any cells until you use the oil immersion objective. Which type of microorganism is most likely?
3. In a wet mount of the scrapings, you observe long filaments composed of many cells. Which type of microorganism is most likely?

EXERCISE 1

Use and Care of the Microscope

The most important DISCOVERIES *of the laws, methods and progress of nature have nearly always sprung from the* EXAMINATION *of the smallest objects which she contains.* – JEAN BAPTISTE LAMARCK

OBJECTIVES

After completing this exercise, you should be able to:

1. Demonstrate the correct use of a compound light microscope.
2. Name the major parts of a compound microscope.
3. Determine the relative sizes of different microbes.
4. Identify the three basic morphologies of bacteria.

BACKGROUND

Virtually all organisms studied in microbiology are invisible to the naked eye and require the use of optical systems for magnification. The microscope was invented shortly before 1600 by Zacharias Janssen of the Netherlands. The microscope was not used to examine microorganisms until the 1680s, when a clerk in a dry-goods store, Antoni van Leeuwenhoek, examined scrapings of his teeth and any other substances he could find. The early microscopes, called **simple microscopes,** consisted of biconvex lenses and were essentially magnifying glasses. (See the photograph on page 8.) To see microbes requires a compound microscope, which has two lenses between the eye and the object. This optical system magnifies the object, and an illumination system (sun and mirror or lamp) ensures that adequate light is available for viewing. A **brightfield compound microscope,** which shows dark objects in a bright field, is used most often.

The Microscope

You will be using a brightfield compound microscope similar to the one shown in FIGURE 1.1a. The basic frame of the microscope consists of a **base,** a **stage** to hold the slide, an **arm** for carrying the microscope, and a **body tube** for transmitting the magnified image. The stage may have two clips or a movable mechanical stage to hold the slide. The light source is in the base. Above the light source is a **condenser,** which consists of several lenses that concentrate light on the slide by focusing it into a cone, as shown in FIGURE 1.1b. The condenser has an **iris diaphragm,** which controls the angle and size of the cone of light. This ability to control the *amount* of light ensures that optimal light will reach the slide. Above the stage, on one end of the body tube, is a revolving nosepiece holding three or four **objective lenses.** At the upper end of the tube is an **ocular** or **eyepiece lens** (10× to 12.5×). If a microscope has only one ocular lens, it is called a **monocular** microscope; a **binocular** microscope has two ocular lenses.

Focusing the Microscope

By moving the lens closer to the slide or the stage closer to the objective lens, using the coarse- or fine-adjustment knob, one can focus the image. The larger knob, the **coarse adjustment,** is used for focusing with the low-power objectives (4× and 10×), and the smaller knob, the **fine adjustment,** is used for focusing with the high-power and oil immersion lenses. The coarse-adjustment knob moves the lenses or the stage longer distances. The area seen through a microscope is called the **field of vision.**

The **magnification** of a microscope depends on the type of objective lens used with the ocular. Compound microscopes have three or four objective lenses mounted on a nosepiece: scanning (4×), low-power (10×), high-dry (40× to 45×), and oil immersion (97× to 100×). The magnification provided by each lens is stamped on the barrel. The total magnification of the object is calculated by multiplying the magnification of the ocular (usually 10×) by the magnification of the objective lens. The most important lens in microbiology is the **oil immersion lens;** it has the highest magnification (97× to 100×) and must be used with immersion oil. Optical systems could be built to magnify much more than the 1000× magnification of your microscope, but the resolution would be poor.

The Light Source

Compound microscopes require a light source. The light may be reflected to the condenser by a mirror under the stage. If your microscope has a mirror, the sun or a lamp may be used as the light source. Most compound microscopes have a built-in illuminator in