Laboratory Exercises in

Microbiology

Third Edition

Harley • Prescott

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

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PREFACE

There are many excellent microbiology laboratory manuals on the market and many others that are called "in-house" productions because they are written for a microbiology course at a particular school. Why another microbiology manual? The answer is straightforward. Many instructors want a manual that is directly correlated with a specific textbook. As a result, this laboratory manual was designed and written to be used in conjunction with the textbook *Microbiology,* third edition, by Lansing M. Prescott, John P. Harley, and Donald A. Klein; however, it can be used with other textbooks with slight adaptation.

Since this manual correlates many of the microbiological concepts in the textbook with the various exercises, comprehensive introductory material is not given at the beginning of each exercise. Instead, just enough specific explanation is given to complement, augment, reinforce, and enhance what is in the textbook. We feel that time allocation is an important aspect of any microbiology course. Students should not be required to reread in the laboratory manual an in-depth presentation of material that has already been covered satisfactorily in the textbook.

Each exercise has been designed to be modular and short. This will allow the instructor to pick and choose only those exercises or parts of exercises that are applicable to a specific course. Several exercises usually can be completed in a two- or three-hour laboratory period. The exercises have also been designed to use commonly available equipment, with the least expense involved, and to be completed in the shortest possible time period.

Considering the above parameters, the purpose of this laboratory manual is to guide students through a process of development of microbiological technique, experimentation, interpretation of data, and discovery in a manner that will complement the textbook and make the study of microbiology both exciting and challenging. According to an old Chinese proverb:

> Tell me and I will forget.
> Show me and I might remember.
> Involve me and I will understand.

These words convey our basic philosophy that it is experiences in the microbiology laboratory and the scientific method that help develop students' critical thinking and creativity, and that increase their appreciation of the mechanisms by which microbiologists analyze information. The laboratory accomplishes this by having students become intensely and personally involved in the knowledge they acquire.

The array of exercises was chosen to illustrate the basic concepts of general microbiology as a whole and of the individual applied fields. The protocols vary in content and complexity, providing the instructor with flexibility to mold the laboratory syllabus to the particular needs of the students, available time and equipment, and confines and scope of the course. Furthermore, it provides a wide spectrum of individual exercises suitable for students in elementary and advanced general microbiology as well as those in various allied health programs.

This manual consists of 80 exercises arranged into 17 major parts covering the following basic topics:

PART ONE, Microscopic Techniques, introduces the students to the proper use and care of the different types of microscopes used in the microbiology laboratory for the study of microorganisms.

PART TWO, Bacterial Morphology and Staining, presents the basic procedures for visualization and differentiation of microorganisms based on cell form and various structures.

PART THREE, Basic Laboratory and Culture Techniques, acquaints students with proper laboratory procedures in preparing

microbiological media, and culture techniques that are used in isolating microorganisms.

PART FOUR, Biochemical Activities of Bacteria, introduces some of the biochemical activities that may be used in characterizing and identifying bacteria.

PART FIVE, Rapid Multitest Systems, acquaints students with some of the multitest systems that can be used to identify bacteria.

PART SIX, Unknown Identification, contains two exercises that guide students through the use of *Bergey's Manual of Systematic Bacteriology* in the identification of unknown bacteria.

PART SEVEN, Environmental Factors Affecting Growth of Microorganisms, acquaints students with some of the various physical and chemical agents that affect microbial growth.

PART EIGHT, NINE, and TEN are concerned with the sanitary aspects of water, milk, and food.

PART ELEVEN, Bacteriophages, acquaints students with various aspects of bacteriophages and their use in the laboratory.

PART TWELVE, Immunologic Techniques, acquaints students with the various clinical screening methods used for the isolation and identification of microorganisms.

PART THIRTEEN, Medical Microbiology, presents an overview of some pathogenic microorganisms and acquaints students with basic procedures used in isolation and identification of pathogens from infected hosts, including those from the student's own body.

PART FOURTEEN, Survey of Cyanobacteria and Selected Eucaryotic Microorganisms, presents an overview that is intended to help students appreciate the morphology, taxonomy, and biology of the fungi, protozoa, and algae.

PART FIFTEEN, Soil Microbiology, provides experience with isolating microorganisms from the soil and selected aspects of the nitrogen cycle.

PART SIXTEEN, Microbial Genetics, presents three experiments designed to illustrate the general principles of bacterial genetics.

PART SEVENTEEN, The Scientific Method, presents one exercise on the demonstration of Koch's postulates helps the student understand the various steps in the scientific method. This exercise can also be used as an investigative type experiment.

The format of each exercise in this manual is intended to promote learning and mastery in the shortest possible time. To this end, each experiment is designed as follows:

Safety Considerations

This laboratory manual endeavors to include many of the safety precautionary measures established by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia; the Occupational Safety and Health Administration (OSHA); and the Environmental Protection Agency (EPA). Efforts are made to instruct the student on safety, and all exercises will contain precautionary procedures that the above agencies are enforcing in hospitals, nursing homes, commercial laboratories, and industry. A **safety considerations box** is included for each exercise to help both the instructor and student prepare themselves for the possibility of accidents.

Both the instructor and student should keep in mind at all times that most technical programs, such as a microbiology laboratory, carry some measure of associated risk. The microbiology laboratory is a place where infectious microorganisms are handled, examined, and studied with safety and effectiveness. However, any of the microorganisms we work with may be pathogenic in an immunocompromised person. Therefore, rather than modifying the objectives in this laboratory manual to avoid any risk, the authors propose that instructors and students implement the Centers for Disease Control and Prevention (CDC) principles of biosafety throughout. One way we propose is to simply modify the "Universal Precautions" (*see front and back covers of this manual*) so the wording is appropriate for the classroom by simply changing "laboratory worker" to "student." In addition, a written safety policy consistent with CDC guidelines and adopted by your institution's governing body will protect you, your institution, and the students. As in any laboratory, safety should be a major part of the curriculum. Students should be required to demonstrate their knowledge of safety before they begin each laboratory exercise.

Materials per Student or Group of Students

To aid in the preparation of all exercises, each procedure contains a list of the required cultures with American Type Culture Collection catalog numbers (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 29852–1776), media, reagents, and other equipment necessary to complete the exercise in the allocated lab time either per student or group of students. Appendixes *I* and *J* provide recipes for reagents, stains, and culture media. Appendix *K* describes the maintenance of microorganisms and supply sources.

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Learning Objectives

Each exercise has a set of learning objectives that define the specific goals of the laboratory session. It is to the student's advantage to read through this list before coming to class. In like manner, these objectives should be given special attention during the laboratory exercise. Upon conscientious completion of the exercise, the student should be able to meet all of the objectives for that exercise. Before leaving the class, students should check the objectives once again to see that they can master them. If problems arise, consult the instructor.

Suggested Reading in Textbook

These cross-references have been designed to save the student's time. By referring the student to sections, paragraphs, tables, charts, figures, and boxes within the textbook, unnecessary duplication is avoided.

Pronunciation Guide

This section contains the phonetic pronunciations for all organisms used in the exercise. If students take the time to sound out new and unfamiliar terms and say them aloud several times, they will learn to use the vocabulary of microbiologists.

Why Are the Above Bacteria, Slides, or Other Microorganisms Used in This Experiment?

The authors have chosen specific viruses, bacteria, fungi, protozoa, algae, and various prepared slides for each exercise. This microbial material has been selected based on cost, ease of growth, availability, reliability, and most importantly, the ability to produce the desired experimental results. In order to communicate these guidelines to the student, this section explains why the authors have chosen the microbial material being used and also gives additional biochemical, morphological, and taxonomic information about the microorganism(s) that the student should find helpful when performing the experiment.

Principles

This section contains a brief discussion of the microbiological principles, concepts, and techniques that underlie the experimental procedures being performed in the exercise.

Procedure

Explicit instructions are augmented by diagrams to aid students in executing the experiment as well as interpreting the results. Where applicable, actual results are shown so that the student can see what should be obtained.

Hints and Precautions

Additional information on what to watch out for, what can go wrong, and helpful tidbits to make the experiment work properly are presented in accompanying boxes.

Laboratory Report

Various pedagogical techniques are used for recording the obtained results. This part of the exercise can be turned in to the instructor for checking or grading.

Review Questions

Review questions are located at the end of each laboratory report. These were written so that students can test their understanding of the concepts and techniques presented in each exercise.

Dilution Ratios Used in This Manual

According to the *American Society for Microbiology Style Manual,* dilution ratios may be reported with either colons (:) or shills (/), but note there is a difference between them. A shill indicates the ratio of a part to a whole; e.g., ½ means 1 of 2 parts, with a total of 2 parts. A colon indicates the ratio of 1 part to 2 parts, with a total of 3 parts. Thus, ½ equals 1:1, but 1:2 equals ⅓.

Instructor's Guide

An instructor's guide has been prepared for the laboratory manual. If you do not have a copy of this guide, you can obtain one free from the publisher. This guide will aid you in preparing the laboratory experiments for the students as well as saving time in preparation.

Finally, it is our hope that this manual will serve as a vehicle to (1) introduce the complexity and diversity of microorganisms and their relationships to one another; (2) provide a solid foundation for further study for those electing a career in science; and (3) convey something of the meaning, scope, and excitement of microbiology as a significant perspective from which to view the world.

We appreciate the many comments offered to us over the years by both faculty and students. In our desire to continue to improve this laboratory manual, we invite constructive comments from those using it. Please contact us through the Cell and Molecular Biology Editor, Wm. C. Brown Publishers.

John P. Harley
Lansing M. Prescott

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ORIENTATION TO THE LABORATORY: RULES OF CONDUCT AND GENERAL SAFETY

Many of the microorganisms used in this course may be pathogenic for humans and animals. As a result, certain rules are necessary to avoid the possibility of infecting yourself or other people. Anyone who chooses to disregard these rules or exhibits carelessness that endangers others may be subject to immediate dismissal from the laboratory. If doubt arises as to the procedure involved in handling infectious material, consult your instructor. Each student is responsible for the observance of the following rules:

1. Place all extra clothing, unnecessary books, purses, backpacks, and paraphernalia in an appropriate place. Racks are provided for these materials. The laboratory work area must be kept free of articles not actually in use.
2. Eating, drinking, and smoking are forbidden at all times in the laboratory.
3. Keep your locker or laboratory drawer clean. Do not allow your locker or drawer to become filled with cultures that have no value in your current work.
4. Return all reagents, cultures, and glassware to their proper places.
5. Wear a laboratory coat, smock, or lab apron when working in the laboratory. This will protect clothing from contamination or accidental discoloration by staining solutions.
6. Do not place anything in your mouth while in the laboratory. This includes pencils, food, and fingers. Learn to keep your hands away from your mouth and eyes.
7. Avoid contamination of the benches, floor, and wastebaskets.
8. Clean your work area (laboratory bench) with a phenolic disinfectant such as 5% Lysol or 5% phenol or a quaternary compound such as cetylpyridinium chloride (Ceepyrn) before and after each laboratory period. This standard procedure lessens the chance for accidental infection as well as for contamination of cultures.
9. Special receptacles will be provided for infectious materials and used glass slides. Place all discarded cultures and contaminated glassware into these receptacles. Do not let unwanted and unneeded materials accumulate. Tall jars filled with a solution such as 5% Lysol or special receptacles will be provided for pipettes.
10. When infectious material is accidentally spilled, cover it immediately with a disinfectant such as 5% Lysol or 5% phenol and notify your instructor at once.
11. Flame wire loops and needles before and immediately after transfer of cultures. Do not move through the laboratory with a loop or pipette containing infectious material.
12. Wash your hands thoroughly before and after each experiment, using disinfectant soap if possible.
13. Label all experimental material with your

a. Name	M. Porter
b. Date	1/18/96
c. Exercise number	Ex. 5
d. Lab section	8–10 M
e. Specimen/ Organism	Water/ *E. coli*

14. Long hair should be tied back to minimize fire hazard and contamination of experiments and cultures.
15. Some of the chemicals employed in the various exercises can be hazardous if not handled properly. We have selected experiments to minimize the use of such chemicals; however, where they are necessary, be certain to observe the precautions noted in the exercise and by your instructor.
16. Do not stack petri plates more than three high on incubator shelves. Tall stacks are a potential hazard if they topple when the incubator is opened. If available, special petri plate holders (cans) may be used to hold large stacks of plates.
17. To avoid burns, beware of Bunsen burners that will be used in almost every exercise. Immediately report all cuts and injuries to your instructor.
18. The Workplace Hazardous Materials Information System (WHMIS) requires that all hazardous substances, including microorganisms, be labeled in a specific manner. In addition, there must be a Material Safety Data Sheet (MSDS) available to accompany each hazardous substance. MSDS sheets are now supplied with every chemical sold by supply houses. The person in charge of the microbiology laboratory should ensure that adherence to this law is enforced.
19. Finally, note where all the specific safety features are located in the laboratory. These would include the fire extinguishers, the safety shower, the fire blanket, the eye wash station, the first aid kit, and emergency exit. If the instructor does not supply this information, please ask him or her to do so.

All laboratory work can be done more effectively and efficiently if the subject matter is understood before coming to the laboratory. To accomplish this, read the experiment several times before the laboratory begins. Know how each exercise is to be done and what principles it is intended to convey. Also, read the appropriate sections in your textbook that pertain to the experiment being performed. This will save you much time and effort during the actual laboratory period.

All laboratory experiments will begin with a brief discussion by your instructor of what is to be done, the location of materials, and other important information. Feel free to ask questions if you do not understand the instructor or the principles involved.

Much of the work in the laboratory is designed to be carried out in groups or with a partner. This is to aid in coverage of subject matter, to save on time and expense, and to encourage discussion of data and results.

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PART ONE

Microscopic Techniques

Microbiologists employ a variety of light microscopes in their work: bright-field, dark-field, phase-contrast, and fluorescence are most commonly used. In fact, the same microscope may be a combination of types: bright-field and phase-contrast, or phase-contrast and fluorescence. You will use these microscopes and the principles of microscopy extensively in this course as you study the form, structure, staining characteristics, and motility of different microorganisms. Therefore, proficiency in using the different microscopes is essential to all aspects of microbiology and must be mastered at the very beginning of a microbiology course. The next five exercises have been designed to accomplish this major objective.

Anton van Leeuwenhoek (1632–1723).

Leeuwenhoek was a master at grinding lenses for his microscopes. Working in Delft Holland, in the mid-1600s, he is considered the greatest early microscopist.

Leeuwenhoek was a manic observer, who tried to look at everything with his microscopes.

> Those little animals were everywhere! He told the Royal Society of finding swarms of those subvisible things in his mouth—of all places: "Although I am now fifty years old," he wrote, "I have uncommonly well-preserved teeth, because it is my custom every morning to rub my teeth very hard with salt, and after cleaning my teeth with a quill, to rub them vigorously with a cloth. . . ."
>
> From his teeth he scraped a bit of white stuff, mixed it with pure rainwater, stuck it in a little tube onto the needle of his microscope, closed the door of his study—
>
> As he brought the tube into focus, there was an unbelievable tiny creature, leaping about in the water of the tube. . . . There was a second kind that swam forward a little way, then whirled about suddenly, then tumbled over itself in pretty somersaults. . . . There was a menagerie in his mouth! There were creatures shaped like flexible rods that went to and fro . . . there were spirals that whirled through the water like violently animated corkscrews. . . .
>
> —Paul Kruif
> *Microbe Hunters* (1926)

EXERCISE 1

Bright-Field Light Microscope

SAFETY CONSIDERATIONS
Slides and coverslips are glass. Be careful with them. Do not cut yourself when using them. The coverslips are very thin and easily broken. Dispose of any broken glass in the appropriately labeled container.

Materials per Student

compound microscope
lens paper
immersion oil
prepared stained slides of several types of bacteria (rods, cocci, spirilla)
glass slides
coverslips
dropper with bulb
newspaper or cut out letter *e*'s
tweezers

Learning Objectives

Each student should be able to:

1. Identify all the parts of a compound microscope
2. Know how to correctly use the microscope—especially the oil immersion lens
3. Learn how to make and examine a wet-mount preparation

Suggested Reading in Textbook

1. The Bright-Field Microscope, chapter 2; see also figures 2.3–2.6.

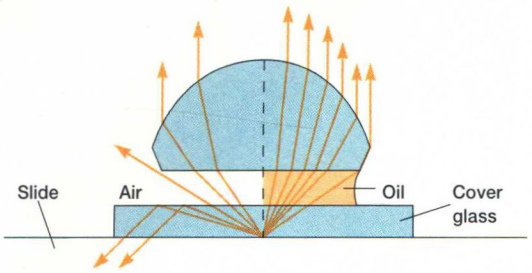
Why Are Prepared Slides of Rods, Cocci, and Spirilla Used in This Exercise?

Because this is a microbiology course and most of the microorganisms studied are bacteria, this is an excellent place to introduce the student to the three basic bacterial shapes: cocci, rods, and spirilla. By gaining expertise in using the bright-field light microscope, the student should be able to observe these three bacterial shapes by the end of the lab period. In addition, the student will gain an appreciation for the small size and arrangement of procaryotic cell structure.

Principles

The **bright-field light microscope** is an instrument that magnifies images using two lens systems. Initial magnification occurs in the **objective lens.** Most microscopes have at least three objective lenses on a rotating base, and each lens may be rotated into alignment with the **eyepiece** or **ocular lens** in which the final magnification occurs. The objective lenses are identified as the **low-power, high-dry,** and **oil immersion objectives.** Each objective is also designated by other terms. These terms give either the **linear magnification** or the **focal length.** The latter is about equal to or greater than the **working distance** between the specimen when in focus and the tip of the objective lens. For example, the low-power objective is also called the **10×,** or **16 millimeter (mm), objective;** the high-dry is called the **40×,** or **4 mm, objective;** and the oil immersion is called the **90×, 100×,** or

Figure 1.1 The Oil Immersion Objective. An oil immersion objective lens operating in air and with immersion oil. Light rays that must pass through air are bent (refracted), and many do not enter the objective lens. The immersion oil prevents the loss of light rays.



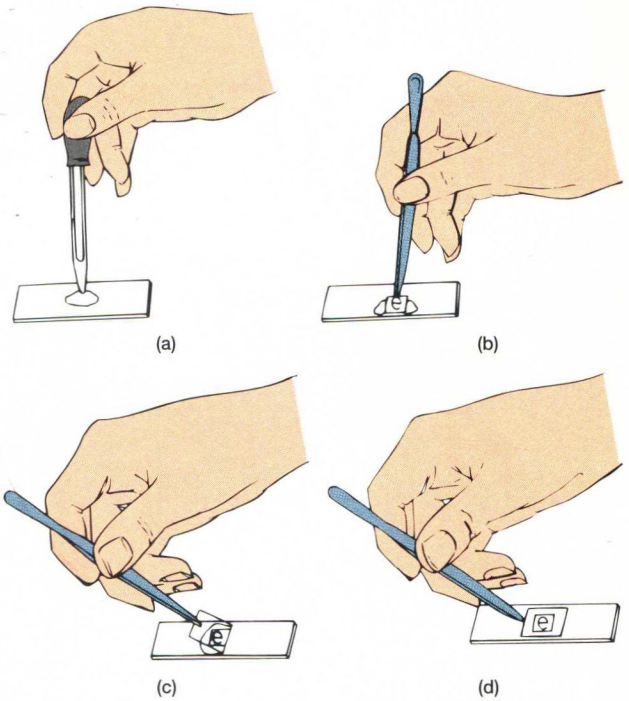
1.8 mm, objective. As the magnification increases, the size of the lens at the tip of the objective becomes progressively smaller and admits less light. This is one of the reasons that changes in position of the **substage condenser** and **iris diaphragm** are required when using different objectives if the specimens viewed are to be seen distinctly. The condenser focuses the light on a small area above the stage, and the iris diaphragm controls the amount of light that enters the condenser. When the oil immersion lens is used, immersion oil fills the space between the objective and the specimen. Because immersion oil has the same **refractive index** as glass, the loss of light is minimized (figure 1.1). The **eyepiece** or **ocular** at the top of the tube magnifies the image formed by the objective lens. As a result, the total magnification seen by the observer is obtained by multiplying the magnification of the objective lens by the magnification of the ocular or eyepiece. For example, when using the 10× ocular and the 43× objective, total magnification is $10 \times 43 = 430$ times.

Procedure

Proper Use of the Microscope

1. Always carry the microscope with two hands. Place it on the desk with the open part away from you.
2. Cut a lowercase *e* from a newspaper or other printed page. Prepare a wet-mount as illustrated in figure 1.2. Place the glass slide on the stage of the microscope and secure it firmly using stage clips. If your microscope has a mechanical stage device, place the slide securely in it. Move the slide until the letter *e* is over the opening in the stage.

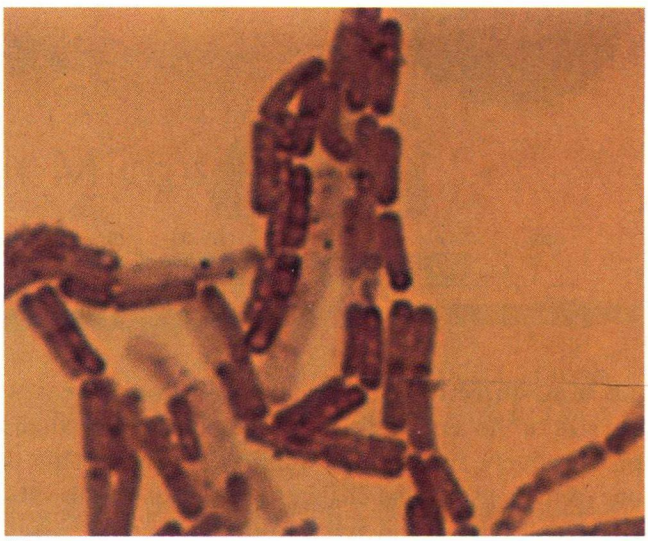
Figure 1.2 Preparation of a Wet-mount Slide. (*a*) Add a drop of water to a slide. (*b*) Place the specimen (letter *e*) in the water. (*c*) Place the edge of a coverslip on the slide so that it touches the edge of the water. (*d*) Slowly lower the coverslip to prevent forming and trapping air bubbles.



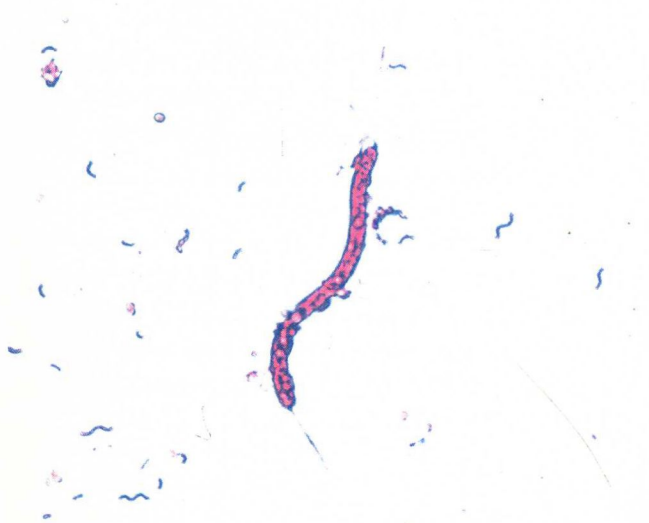
3. With the low-power objective in position, lower the tube until the tip of the objective is within 5 mm of the slide. Be sure that you lower the tube while looking at the microscope from the side.
4. Look into the microscope and slowly raise the tube by turning the coarse adjustment knob counterclockwise until the object comes into view. Once the object is in view, use the fine adjustment knob to focus the desired image.
5. Open and close the diaphragm, and lower and raise the condenser, noting what effect these actions have on the appearance of the object being viewed. Usually the microscope is used with the substage condenser in its topmost position. The diaphragm should be open and then closed down until just a slight increase in contrast is observed.
6. Use the oil immersion lens to examine the stained bacteria that are provided (figure 1.3*a–d*). The directions for using this lens are as follows: First locate the stained area with the low-power objective and then turn the oil immersion lens into the oil and focus with the fine adjustment. An alternate procedure is to get the focus very sharp under high power, then move the revolving nosepiece until you are halfway between the high-power and oil

Figure 1.3 Examples of Bacterial Shapes as Seen with the Bright-field Light Microscope. (*a*) *Staphylococcus aureus* cocci (×1,000). (*b*) *Bacillus subtilis* rods or bacilli (×1,000). (*c*) A single, large spirillum (*Spirillum volutans;* ×1,000). (*d*) Numerous, small spirilla (*Rhodospirillum rubrum;* ×1,000).

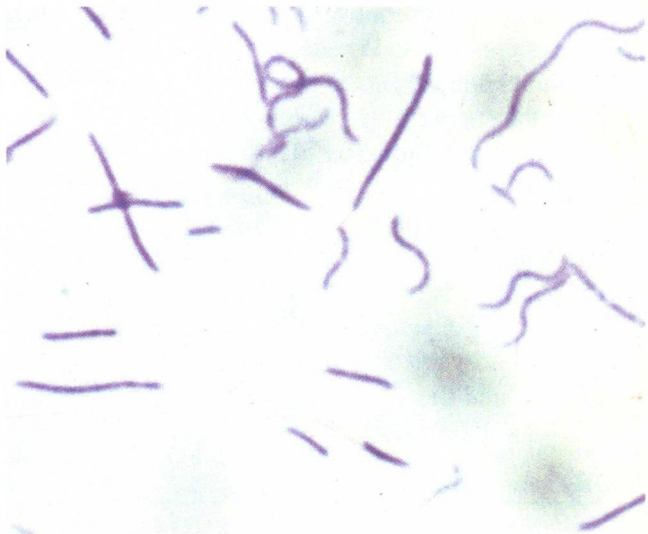
(a)



(b)

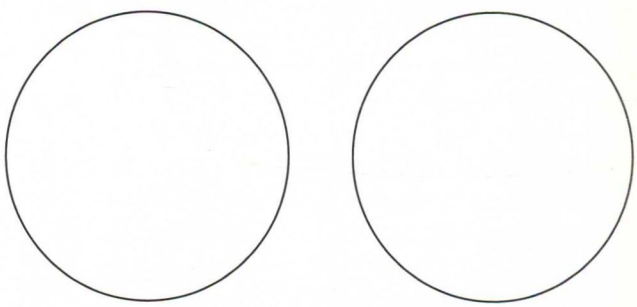


(c)



(d)

immersion objectives. Place a small drop of immersion oil in the center of the illuminated area on the slide. Continue revolving the nosepiece until the oil immersion objective clicks into place. The lens will now be immersed in oil. Sharpen the focus with the fine adjustment knob. Draw a few of the bacteria in the space to the right.



7. After you are finished with the microscope, place the low-power objective in line with the ocular, lower the tube to its lowest position, clean the oil from the oil immersion lens, cover, and return the microscope to its proper storage place.

HINTS AND PRECAUTIONS

(1) Forcing the fine or coarse adjustment knobs on the microscope beyond their gentle stopping points can render the microscope useless. (2) A general rule for you to note is that the lower the magnification, the less light should be directed upon the object. (3) The fine adjustment knob on the microscope should be centered prior to use to allow for maximum adjustment in either direction. (4) If a slide is inadvertently placed upside down on the microscope stage, you will have no difficulty focusing the object under low and high power. However, when progressing to oil immersion, you will find it impossible to bring the object into focus. (5) Slides should always be placed on and removed from the stage when the low power (4× or 10×) objective is in place. Removing a slide when the higher objectives are in position may scratch the lenses.

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