Harley
Prescott

fifth edition

ry Exercises in

MICROBIOLOGY

Laboratory Exercises in

Microbiology

Fifth Edition

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PREFACE

Take interest, I implore you, in those sacred dwellings which one designates by the expressive term: laboratories. Demand that they be multiplied, that they be adorned. These are the temples of the future—temples of well-being and of happiness. There it is that humanity grows greater, stronger, better.

Louis Pasteur
(French chemist, founder of microbiology, 1822–1895)

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,* fifth 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.

In 1997, the American Society for Microbiology, through its Office of Education and Training, adopted a Laboratory Core Curriculum representing themes and topics considered essential to teach in every introductory microbiology laboratory, regardless of its emphasis. An instructor might add items appropriate to allied health, applied, environmental, or majors microbiology courses.

The Laboratory Core is not meant to be a syllabus or outline. The core themes and topics are meant to frame objectives to be met somewhere within the introductory microbiology laboratory. Depending on the

specific emphasis of the course, a single lab session could meet multiple core objectives, focus on one objective, or emphasize a topic that is not in the lab core but is important to that particular course.

Laboratory Skills

A student successfully completing basic microbiology will demonstrate the ability to

1. **Use a bright-field light microscope** to view and interpret slides, including
    a. correctly setting up and focusing the microscope
    b. proper handling, cleaning and storage of the microscope
    c. correct use of all lenses
    d. recording microscopic observations
2. **Properly prepare slides** for microbiological examination, including
    a. cleaning and disposal of slides
    b. preparing smears from solid and liquid cultures
    c. performing wet-mount and/or hanging drop preparations
    d. performing Gram stains
3. **Properly use aseptic techniques** for the transfer and handling of microorganisms and instruments, including
    a. sterilizing and maintaining sterility of transfer instruments
    b. performing aseptic transfer
    c. obtaining microbial samples
4. **Use appropriate microbiological media and test systems,** including
    a. isolating colonies and/or plaques
    b. maintaining pure cultures
    c. using biochemical test media
    d. accurately recording macroscopic observations
5. **Estimate the number of microorganisms** in a sample using serial dilution techniques, including
    a. correctly choosing and using pipettes and pipetting devices
    b. correctly spreading diluted samples for counting
    c. estimating appropriate dilutions
    d. extrapolating plate counts to obtain correct CFU or PFU in the starting sample
6. **Use standard microbiology laboratory equipment correctly,** including
    a. using the standard metric system for weights, lengths, diameters, and volumes
    b. lighting and adjusting a laboratory burner
    c. using an incubator

Laboratory Thinking Skills

A student successfully completing basic microbiology will demonstrate an increased skill level in

1. **Cognitive processes,** including
    a. formulating a clear, answerable question
    b. developing a testable hypothesis
    c. predicting expected results
    d. following an experimental protocol
2. **Analysis skills,** including
    a. collecting and organizing data in a systematic fashion
    b. presenting data in an appropriate form (graphs, tables, figures, or descriptive paragraphs)
    c. assessing the validity of the data (including integrity and significance)
    d. drawing appropriate conclusions based on the results
3. **Communications skills,** including
    a. discussing and presenting laboratory results or findings in the laboratory
4. **Interpersonal and citizenry skills,** including
    a. working effectively in groups or teams so that the task, results, and analysis are shared
    b. effectively managing time and tasks to be done simultaneously, by individuals and within a group
    c. integrating knowledge and making informed judgments about microbiology in everyday life

Laboratories typically supplement and integrate closely with the lecture content in ways that are unique to each instructor. Consequently, the laboratory content that is considered essential for laboratory work by one instructor may be covered in lecture portion of the course by another instructor, making it difficult to define specific top-

ics that should be integral in all microbiology laboratories. As a result, the ASM Laboratory Core Curriculum Committee developed themes, which are broadly based and will enable instructors to have the flexibility to use a wide variety of laboratories to meet the suggested core.

A student successfully completing basic microbiology will demonstrate mastery of the basic principles of the following themes and complete laboratory activities that focus on one or more of the topics under each theme.

Theme 1. Integrating themes—impact of microorganisms on the biosphere and humans; microbial diversity

Theme 2. Microbial cell biology, including cell structure and function, growth and division, and metabolism

Theme 3. Microbial genetics, including mutations

Theme 4. Interactions of microorganisms with hosts (humans, other animals, plants), including pathogenicity mechanisms and antimicrobial agents

In order to meet the above themes, topics, and skills (The American Society for Microbiology Laboratory Core Curriculum), this manual consists of 66 exercises arranged into 11 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 in 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, Environmental and Food Microbiology, is concerned with the environmental aspects of water, milk, and food.

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

PART ELEVEN, Microbial Genetics and Genomics, presents six experiments designed to illustrate the general principles of bacterial genetics and genomics.

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 these 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 pp. xiii–xiv*) 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; *www.ATCC.org;* 703-365-2700), media, reagents, and other equipment necessary to complete the exercise in the allocated lab time either per student or group of students. Appendixes H and I provide recipes for reagents, stains, and culture media. Appendix J describes the maintenance of microorganisms and supply sources.

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.

Medical Application

Many students using this laboratory manual are either in one of the allied health disciplines, such as nursing, or in a preprofessional program such as premed, predent, or prevet and need to know the clinical relevance of each exercise performed. To satisfy this need, a *Medical Application* section is included for some of the medically oriented exercises. Medical applications are described for most clinical procedures as a specific application of the purpose of the exercise. For example, a procedure can be used for the identification of a particular microorganism or used in combination with other exercises in a diagnosis. For these exercises, some important pathogens with their diseases and their need for the test being performed in the exercise are listed.

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.

Dilution Problems

Since dilution problems are such an integral part of any microbiology course, *Appendix A* gives an overview of the different types of dilution. This includes a variety of practice problems. Answers are provided.

Instructor's Guide

An instructor's guide has been prepared for the laboratory manual and is available on our web site at **www.mhhe.com/prescott5.** This guide provides answers to the questions in this manual.

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, McGraw-Hill Publishers **(www.mhhe.com/prescott5).**

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.

In 1997, the American Society for Microbiology, through its Office of Education and Training, adopted the following on laboratory safety. Each point is considered essential for every introductory microbiology laboratory, regardless of its emphasis.

A student successfully completing basic microbiology will demonstrate the ability to explain and practice safe

1. **Microbiological procedures,** including
    a. reporting all spills and broken glassware to the instructor and receiving instructions for cleanup
    b. methods for aseptic transfer
    c. minimizing or containing the production of aerosols and describing the hazards associated with aerosols
    d. washing hands prior to and following laboratories and at any time contamination is suspected
    e. never eating or drinking in the laboratory
    f. using universal precautions (see inside front and end covers of this laboratory manual)
    g. disinfecting lab benches prior to and at the conclusion of each lab session
    h. identification and proper disposal of different types of waste
    i. never applying cosmetics, including contact lenses, or placing objects (fingers, pencils) in the mouth or touching the face
    j. reading and signing a laboratory safety agreement indicating that the student has read and understands the safety rules of the laboratory
    k. good lab practice, including returning materials to proper locations, proper care and handling of equipment, and keeping the bench top clear of extraneous materials
2. **Protective procedures,** including
    a. tying long hair back, wearing personal protective equipment (eye protection, coats, closed shoes; glasses may be preferred to contact lenses), and using such equipment in appropriate situations
    b. always using appropriate pipetting devices and understanding that mouth pipetting is forbidden
3. **Emergency procedures,** including
    a. locating and properly using emergency equipment (eye-wash stations, first-aid kits, fire extinguishers, chemical safety showers, telephones, and emergency numbers)
    b. reporting all injuries immediately to the instructor
    c. following proper steps in the event of an emergency

In addition, institutions where microbiology laboratories are taught will

1. train faculty and staff in proper waste stream management
2. provide and maintain necessary safety equipment and information resources
3. train faculty, staff, and students in the use of safety equipment and procedures
4. train faculty and staff in the use of MSDS. 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.

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 principle 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 the materials, and other important information. Feel free to ask questions if you do not understand the instructor or the principle 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 time and expense, and to encourage discussion of data and results.

Many of the ASM's recommended precautions are represented by the specific safety guidelines given inside the cover of this laboratory manual.

I have read the above rules and understand their meaning

_____

Signature

_____

Date

SUMMARY OF UNIVERSAL PRECAUTIONS AND LABORATORY SAFETY PROCEDURES

Universal Precautions

Since medical history and examination cannot reliably identify all patients infected with HIV or other blood-borne pathogens, blood and body-fluid precautions should be consistently used for all patients.

1. All health-care workers should routinely use appropriate barrier precautions to prevent skin and mucous-membrane exposure when contact with blood or other body fluids of any patient is anticipated. Gloves should be worn for touching blood and body fluids, mucous membranes, or non-intact skin of all patients, for handling items or surfaces soiled with blood or body fluids, and for performing venipuncture and other vascular access procedures. Gloves should be changed after contact with each patient. Masks and protective eyewear or face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids.
2. Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed.
3. All health-care workers should take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments or devices during procedures; when cleaning used instruments; during disposal of used needles; and when handling sharp instruments after procedures. To prevent needlestick injuries, needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand. After they are used, disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers for disposal.
4. Although saliva has not been implicated in HIV transmission, to minimize the need for emergency mouth-to-mouth resuscitation, mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable.
5. Health-care workers who have exudative lesions or weeping dermatitis should refrain from all direct patient care and from handling patient-care equipment.
6. The following procedure should be used to clean up spills of blood or blood-containing fluids: (1) Put on gloves and any other necessary barriers. (2) Wipe up excess material with disposable towels and place the towels in a container for sterilization. (3) Disinfect the area with either a commercial EPA-approved germicide or household bleach (sodium hypochlorite). The latter should be diluted from 1:100 (smooth surfaces) to 1:10 (porous or dirty surfaces); the dilution should be no more than 24 hours old. When dealing with large spills or those containing sharp objects such as broken glass, first cover the spill with disposable toweling. Then saturate the toweling with commercial germicide or a 1:10 bleach solution and allow it to stand for at least 10 minutes. Finally clean as described above.

Precautions for Laboratories

Blood and other body fluids from all patients should be considered infective.

1. All specimens of blood and body fluids should be put in a well-constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.
2. All persons processing blood and body-fluid specimens should wear gloves. Masks and protective eyewear should be worn if mucous-membrane contact with blood or body fluids is anticipated. Gloves should be changed and hands washed after completion of specimen processing.
3. For routine procedures, such as histologic and pathologic studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets should be used whenever procedures are conducted that have a high potential for generating droplets. These include activities such as blending, sonicating, and vigorous mixing.
4. Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done,
5. Use of needles and syringes should be limited to situations in which there is no alternative, and the recommendations for preventing injuries with needles outlined under universal precautions should be followed.
6. Laboratory work surfaces should be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.
7. Contaminated materials used in laboratory tests should be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of infective waste.
8. Scientific equipment that has been contaminated with blood or other body fluids should be decontaminated and cleaned before being repaired in the laboratory or transported to the manufacturer.
9. All persons should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.
10. There should be no eating, drinking, or smoking in the work area.

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PART ONE

Microscopic Techniques

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 Pierre Antoine Monet de Lamarck
(French naturalist, 1744–1829)

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.

After completing at least exercise 1, you will, at the minimum, be able to demonstrate the ability to use a bright-field light microscope. This will meet the American Society for Microbiology Core Curriculum skill number 1 (*see pp. vi–viii*): (a) correctly setting up and focusing the microscope; (b) proper handling, cleaning, and storage of the microscope; (c) correct use of all lenses; and (d) recording microscopic observations.

Antony 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 de Kruif
> *Microbe Hunters* (1926)

EXERCISE 1

Bright-Field Light Microscope and Microscopic Measurement of Organisms

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. If your microscope has an automatic stop, do not use it as the stage micrometer is too thick to allow it to function properly. It may result in a shattered or broken slide or lens.

Materials per Student

compound microscope
lens paper and lens cleaner
immersion oil
prepared stained slides of several types of bacteria (rods, cocci, spirilla), fungi, algae, and protozoa
glass slides
coverslips
dropper with bulb
newspaper or cut-out letter *e*'s
tweezers
ocular micrometer
stage micrometer

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
4. Understand how microorganisms can be measured under the light microscope
5. Calibrate an ocular micrometer
6. Perform some measurements on different microorganisms

Suggested Reading in Textbook

1. The Bright-Field Microscope, section 2.2; see also figures 2.3–2.6.
2. See tables 2.1 and 34.1

Medical Application

In the clinical laboratory, natural cell size, arrangement and motility are important characteristics in the identification of a bacterial pathogen.

Why Are Prepared Slides 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.

One major objective of this exercise is for the student to understand how microorganisms can be measured under the light microscope and to actually perform some measurements on different microorganisms. By making measurements on prepared slides of various bacteria, fungi, algae, and protozoa, the student will gain an appreciation for the size of different microorganisms discussed throughout both the lecture and laboratory portions of this course.

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 magni-**