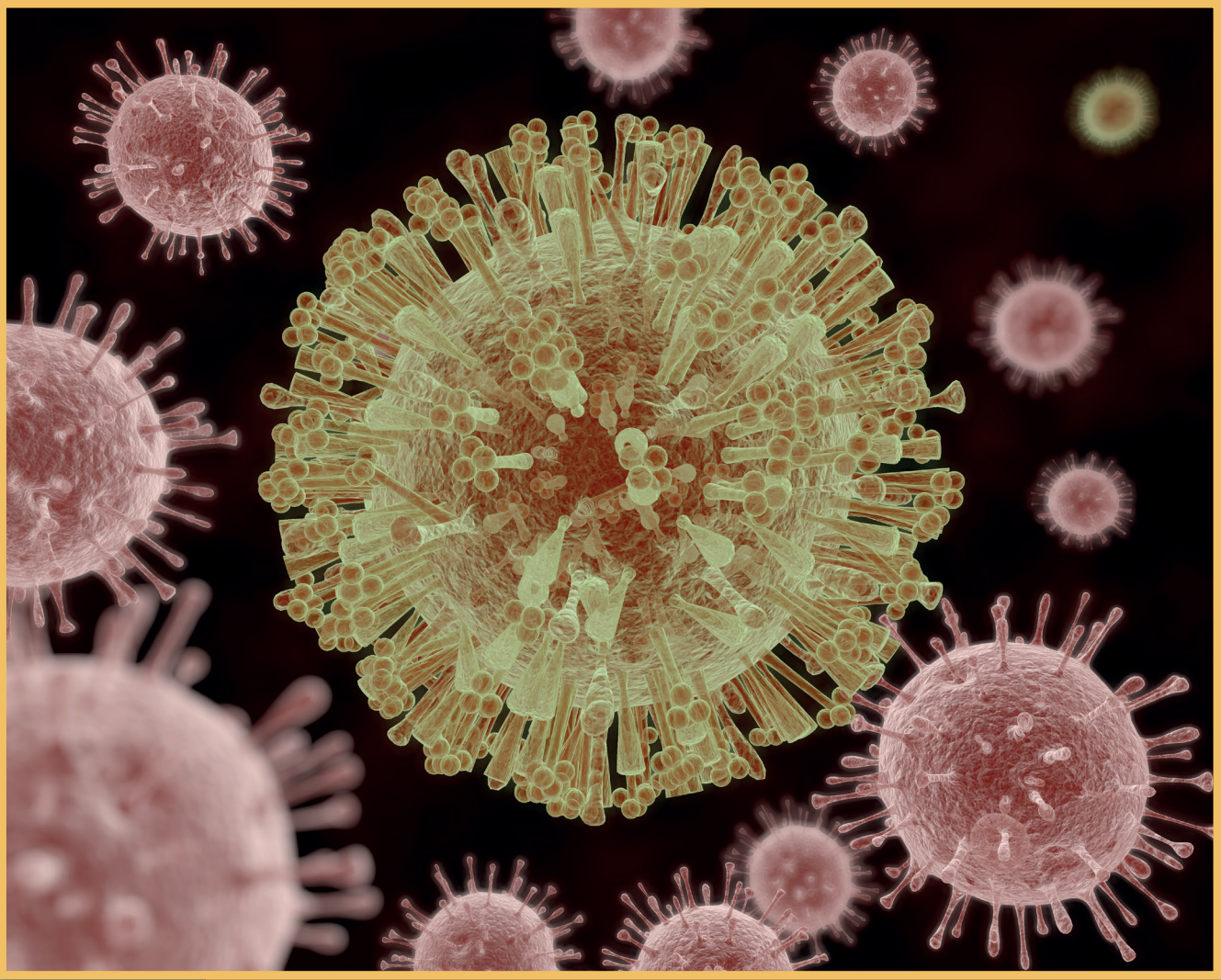
Twelfth Edition



Laboratory Manual and Workbook in MICROBIOLOGY

Applications to Patient Care

Paul A. Granato | Verna Morton | Josephine A. Morello

Mc Graw Hill Education

TWELFTH EDITION

LABORATORY MANUAL AND WORKBOOK IN MICROBIOLOGY

APPLICATIONS TO PATIENT CARE

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Contents

PREFACE

To the Student:

You are about to begin the study of a fascinating group of organisms that are of immense importance in human health and disease. Although infectious diseases were once predicted to disappear with the widespread use of antimicrobial agents, the microbes have retaliated by means of their remarkable genetic versatility. Not only have some species acquired resistance to almost all existing antimicrobial agents but, in part due to social and political unrest, some old diseases have returned. New diseases, even some forms of cancer, are now known to be caused by microbes. Thus, whether your career leads you to direct patient contact as a nurse, or you pursue another patient care responsibility in a field of allied health, knowledge of microbiology is essential to perform your duties optimally and to protect both patients and yourself from acquisition and transfer of sometimes deadly pathogens.

In this context, this laboratory manual leads you through a series of exercises that allow you to learn not only basic techniques for working with microbes similar to those you will encounter in the clinical setting, but they will teach you how to practice safety precautions in the laboratory and hospital environment. Your journey through these experiments will be guided by your instructor, who will determine the time and materials available to perform each exercise. Most likely not every exercise will be performed in your course, but enough of them are available to provide you with a realistic idea of your responsibilities. You will learn what happens to a patient specimen after it is collected for microbiology laboratory analysis and how preliminary and completed laboratory reports are conveyed to the physician.

Several of the procedures you will perform have now been replaced by automated, immunological, or molecular methods, as will be described. However, the exercises in this manual will provide a basic understanding of microbes—their morphology, biochemical activity, immunological characteristics, and susceptibility to antimicrobial agents.

Before each laboratory session, your responsibility is to read through the introductory material of the exercise assigned and the procedures that you will follow, keeping in mind the Learning Objectives that precede each exercise. In this way, you will be able to understand the concepts introduced and complete the work in the time allotted. In some instances, you may work alone, or depending on the instructor and class size, you may work in pairs or groups. In either case, you should be familiar with all procedures and the results of each experiment. At the end of each exercise, you will be asked a series of questions. The question pages are set up so that they can be removed from the manual and handed in to the instructor without destroying the exercises. Be aware that not all answers to the questions are found in the exercise material but will require supplementary reading in your textbook and other literature or a search on the internet. With the rapid pace of medical knowledge, the internet has become a valuable resource to your continuing education, but only if you learn to recognize valid sites, such as those of the Centers for Disease Control and Prevention (www.cdc.gov); the National Library of Medicine (www.nlm.nih.gov, which includes PubMed Central); *Web* MD (www.webmd.com); and ClinLab Navigator (www.clinlabnavigator.com).

During our careers, we authors have been fascinated by the widespread prominence of microbes and their impact on health-related fields. We hope that your introduction to microbiology will increase your appreciation for their importance and need for respectful treatment.

To the Instructor:

This manual, now in its twelfth edition, maintains its original emphasis on the basic principles of diagnostic microbiology for students entering the allied health professions. The authors have emphasized the purposes and function of the clinical microbiology laboratory in the diagnosis of infectious diseases. The exercises illustrate as simply as possible the nature of laboratory

procedures used for isolation and identification of infectious agents, as well as the principles of asepsis, disinfection, and sterilization. With the advent of automated, immunological, and molecular methods, formerly standard techniques for culture identification and antimicrobial susceptibility testing are being replaced by procedures that allow for more rapid test results. These methods are described in each exercise when appropriate, but we believe that the conventional methods practiced herein will help improve students' basic understanding of the morphology, biochemical activity, and immunological characteristics of important microbial pathogens.

Attention is also given to the role of the health professional in regard to appropriate collection of clinical specimens and the applications of aseptic and disinfectant techniques as they relate to patient care. In this way, the student receives the foundation needed to interpret patient-related information for the diagnosis and treatment of infectious diseases.

In this edition of the manual, each exercise has been carefully reviewed for accuracy and currency and revised when necessary to conform to changing practices in clinical laboratories. Two exercises from the eleventh edition, Preparing a Hanging Drop and Pour-Plate Technique, have been deleted because they play little role in current clinical laboratory practice. A description of the new multiplex syndrome panel testing technique using PCR has been added to the description of nucleic acid assays in a new exercise (Exercise 17). The MALDI-TOF instrument, which is gaining widespread use, is described in a new Exercise 18. For details, see What's New in the 12th Edition, which follows. Thought has been given to the time and resources available to instructors and students. Instructors may select among the exercises or parts of exercises they wish to perform, according to the focus of their courses.

A few exercises are primarily descriptive in nature because the equipment, supplies, or reagents needed to perform the relevant tests are beyond the resources available to most teaching laboratories. We considered that the students can benefit from knowing that such techniques are in regular use, even though they may not have the "hands-on" experience. If possible, it would be worthwhile for the instructor to arrange a field trip to a nearby clinical microbiology laboratory so that the students can view these diagnostic procedures firsthand.

Concern has been expressed that not all answers to the questions at the end of each exercise are found in the introductory or procedural material. This omission is purposeful to allow the students to think critically about the lessons and to explore alternative resources, as they will need to do throughout their careers. They should be directed to their textbooks, and in this computer age, to the internet, with caution about possible unreliable sources of information. Government sources such as the Centers for Disease Control and Prevention and the National Library of Medicine (see web addresses in To the Student) are usually accurate, but many other sources are available. The question section of each exercise is set up so that it can be removed from the manual and handed in to the instructor without destroying the exercises.

A complete *Instructor's Manual*, available online from McGraw-Hill Education, should greatly aid instructors' preparation for this course. Included are notes to instructors to help plan each exercise; formulae for preparation of reagents; preparation, storage, and sources of media; and suggested answers to questions in the manual. The URL and password for this site are available from your McGraw-Hill Education representative.

What's New in the 12th Edition

- All figures and colorplates have been carefully reviewed and changes made when necessary.
- In Exercise 3, the hanging drop preparation has been deleted because it is seldom used in clinical practice.
- Because former Exercise 6 (Special Stains) has been deleted from this edition, a brief discussion of capsules, flagella, and endospores has been included in Exercise 5 to orient the student to these bacterial structures.
- In Exercise 6, rather than having students prepare culture media, the media are prepared beforehand by the instructor. However, the students learn how to dispense them aseptically into Petri dishes or in tubes for sterilization.
- In Exercise 8, the pour-plate technique has been deleted because it is seldom used in clinical practice.
- In Exercise 10, conditions of pressure, temperature, and time currently used for steam pressure sterilization in most hospitals have been updated.

- In Exercise 12, a brief description of the problem of multidrug-resistant members of the *Enterobacteriaceae* has been added. The information in table 12.1, Zone Diameter Interpretive Table, has been verified as conforming to the latest Performance Standards of the Clinical and Laboratory Standards Institute.
- Exercise 16 (formerly Exercise 17) is now limited to a discussion of the principles of antigen immunoassays. Although no experiments are performed in this exercise, the students are directed to experiments they will be performing in later exercises.
- The information on nucleic acid assays is now found in Exercise 17, along with a discussion of Multiplex Syndrome Panel Testing Using PCR Assays. This separation from the discussion of antigen immunoassays allows emphasis to be placed on the differences between the technologies and the use of each in the clinical microbiology laboratory.
- Exercise 18 is a new exercise discussing MALDI-TOF Mass Spectrometry for the Rapid Identification of Bacteria and Fungi. This instrument has come into widespread use in the clinical laboratory. The exercise is accompanied by figures describing the instrument and workflow.
- In Exercise 20, the use of PCR for the rapid identification of *Streptococcus pyogenes* in throat specimens is described. The availability of effective vaccines for pneumococcal pneumonia has been added. Figure 20.1 showing latex agglutination has been replaced.
- In Exercise 23, the advantage of molecular gene amplification methods in the detection of enteric pathogens is emphasized.
- In Exercise 26, the importance of the meningococcal vaccine in reducing the incidence of disease has been added.
- In Exercise 27, the importance of microarray assays for rapid identification of bacteria in blood cultures and the detection of antibiotic-resistance genes is described.
- Exercise 30 includes a discussion of the availability of nucleic acid amplification tests for organisms in this exercise. A section on the recent Ebola virus and Zika virus outbreaks has been added.
- Exercise 31 includes the use of nucleic acid and MALDI-TOF MS technologies for yeast identification.

Personalize Your Lab

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P. A. G.

V. M.

J. A. M.

Orientation to the Microbiology Laboratory

Warning

Some of the laboratory experiments included in this text may be hazardous if you handle materials improperly or carry out procedures incorrectly. Safety precautions are necessary when you work with any microorganism and with Bunsen burners, chemicals, glass test tubes, hot water baths, sharp instruments, and similar materials. Your school may have specific regulations about safety procedures that your instructor will explain to you. If you have any problems with materials or procedures, please ask your instructor for help.

Safety Procedures and Precautions

The microbiology laboratory, whether in a classroom or a working diagnostic laboratory, is a place where cultures of microorganisms are handled and examined. This type of activity must be carried out with good aseptic technique in a thoroughly clean, well-organized workplace. In aseptic technique, all materials that are used have been sterilized to kill any microorganisms contained in or on them, and extreme care is taken not to introduce new organisms from the environment. Even if the microorganisms you are studying are not usually considered pathogenic (disease producing), *any* culture of *any* organism should be handled as if it were a potential pathogen. With current medical practices and procedures, many patients with lowered immune defenses survive longer than they did before. As a result, almost any microorganism can cause disease in them under the appropriate circumstances.

Each student must quickly learn and continuously practice aseptic laboratory technique. It is important to prevent contamination of your hands, hair, and clothing with culture material and also to protect your neighbors from such contamination. In addition, you must not contaminate your work with microorganisms from the environment. Once you learn the techniques for asepsis and proper disinfection in the laboratory, they apply to almost every phase of patient care, especially to the collection and handling of specimens that are critical if the laboratory is to make a diagnosis of infectious disease. These specimens should be handled as carefully as cultures so that they do not become sources of infection to others. An important problem in hospitals is the transmission of microorganisms between patients, especially by contaminated hands. Well-trained professionals, caring for the sick, should never be responsible for transmitting infection between patients. Appropriate attention to frequency and method of handwashing (scrubbing with soap for at least 30 seconds) or

use of a hand sanitizing product, as described in Exercise 11, are critical for preventing these hospital-acquired infections (also known as nosocomial infections).

In general, all safety procedures and precautions followed in the microbiology laboratory are designed to:

1. *Restrict microorganisms present in specimens or cultures* to the containers in which they are collected, grown, or studied.
2. *Prevent environmental microorganisms* (normally present on hands, hair, clothing, laboratory benches, or in the air) from entering specimens or cultures and interfering with results of studies.

Hands and bench tops are kept clean with disinfectants, laboratory coats are worn, long hair is tied back, and working areas are kept clear of all unnecessary items. Containers used for specimen collection or culture material are presterilized and capped to prevent entry by unsterile air, and sterile tools are used for transferring specimens or cultures. *Nothing* is placed in the mouth.

Personal conduct in a microbiology laboratory should always be quiet and orderly. The instructor should be consulted promptly whenever problems arise. Any student with a fresh, unhealed cut, scratch, burn, or other injury on either hand should notify the instructor before beginning or continuing with the laboratory work. If you have a personal health problem and are in doubt about participating in the laboratory session, check with your instructor before beginning the work. *Careful attention to the principles of safety is required throughout any laboratory course in microbiology.*

General Laboratory Directions

1. Always read the assigned laboratory material *before* the start of the laboratory period. Pay particular attention to the Learning Objectives at the beginning of each exercise.
2. Before entering the laboratory, remove coats, jackets, and other outerwear. These should be left outside the laboratory, together with any backpacks, books, papers, or other items not needed for the work.
3. To be admitted to the laboratory, each student should wear a fresh, clean, knee-length laboratory coat.
4. At the start and end of each laboratory session, students should clean their assigned bench-top area with a disinfectant solution provided. That space should then be kept neat, clean, and uncluttered throughout each laboratory period.
5. Learn good personal habits from the beginning:
   Tie back long hair neatly, away from the shoulders.
   Do not wear jewelry to laboratory sessions.
   Keep fingers, pencils, and such objects out of your mouth.
   Do not smoke, eat, or drink in the laboratory.
   Do not lick labels with your tongue. Use tap water or preferably, self-sticking labels. Do not wander about the laboratory. Unnecessary activity can cause accidents, distract others, and promote contamination.
6. In the hospital and clinic setting, disposable gloves must be worn when patient care personnel draw blood from patients and when they collect and handle patient specimens. Once culture plates and tubes have been inoculated in the laboratory, however, gloves are not required to perform subsequent procedures.

7. Each student will need bibulous paper, lens paper, a china-marking pencil (or a black, waterproof marking pen) and a 100 mm ruler (purchased or provided). If Bunsen burners are used instead of bacterial incinerators, matches or flint strikers are also needed.
8. Keep a complete record of all your experiments, and answer all questions at the end of each exercise. Your completed work can be removed from the manual and submitted to the instructor for evaluation.
9. Discard all cultures and used glassware into the container labeled *CONTAMINATED* or *BIOHAZARD*. (This container will later be sterilized.) Plastic or other disposable items should be discarded separately from glassware in containers to be sterilized.
   Never place contaminated pipettes on the bench top.
   Never discard contaminated cultures, glassware, pipettes, tubes, or slides in the wastepaper basket or garbage can.
   Never discard contaminated liquids or liquid cultures in the sink.
10. If you are in doubt as to the correct procedure, double-check the manual. If doubt continues, consult your instructor. Avoid asking your neighbor for help with procedures.
11. If you should spill or drop a culture or if any type of accident occurs, *call the instructor immediately.* Place a paper towel over any spill and pour disinfectant over the towel. Let the disinfectant stand for 15 minutes, then clean the spill with fresh paper towels. Remember to discard the paper towels in the proper receptacle and wash your hands carefully.
12. Report any injury to your hands to the instructor either before the laboratory session begins or during the session.
13. Never remove specimens, cultures, or equipment from the laboratory under any circumstances.
14. Before leaving the laboratory, carefully wash and disinfect your hands. Arrange to launder your lab coat so that it will be fresh for the next session.

PART ONE

Basic Techniques of Microbiology

In the study of medical microbiology, we learn to recognize, isolate, and identify those microorganisms that are important in causing human infections and to differentiate them from harmless microorganisms that live in or on the body. We also learn methods to destroy them on animate and inanimate surfaces, such as on hands and in the hospital environment. In addition, we examine methods for guiding physicians in their choice of the most appropriate antimicrobial agent(s) for treating human infections.

In Part One, our study begins with the basic laboratory techniques that are needed to see and work with these microorganisms that are so important in health and disease. Thus, in Exercises 1 through 5, you will gain knowledge about the microscope, procedures for handling and examining cultures, and the staining techniques that enable us to learn about microbial morphology and certain characteristic structures. These steps are the first ones used in the laboratory identification of infectious agents. In Exercises 6 through 8, you will gain an understanding of the composition and use of culture media, which make it possible to grow and further study these microscopic organisms and confirm their role in specific infections. These same techniques are used throughout the study of microbiology, whether related to disease or to the surrounding environment (environmental microbiology).

EXERCISE 1

The Microscope

Learning Objectives

After completing this exercise, students should be able to:

1. Describe the function of the following parts of the microscope:
   ocular lens
   objective lens
   iris diaphragm
   condenser
2. List three things they must do in order to put away their microscopes correctly.
3. Recall the difference between magnification and resolution.
4. State why oil is used on a slide to be examined with the oil-immersion objective.
5. Explain the advantage of parfocal objective lenses.

A good microscope is an essential tool for any microbiology laboratory. There are many kinds of microscopes, but the type most useful in diagnostic work is the *compound microscope.* By means of a series of lenses and a source of bright light, it magnifies and illuminates minute objects such as bacteria and other microorganisms that would otherwise be invisible to the eye. This type of microscope will be used throughout your laboratory course. As you gain experience using it, you will realize how precise it is and how valuable it is for studying microorganisms present in clinical specimens and in cultures. Even though you may not use a microscope in your profession, a firsthand knowledge of how to use it is important. Your laboratory experience with the microscope will give you a lasting impression of living forms that are too small to be seen unless they are highly magnified. As you learn about these "invisible" microorganisms, you should be better able to understand their role in transmission of infection.

Purpose	To study the compound microscope and learn A. Its important parts and their functions B. How to focus and use it to study microorganisms C. Its proper care and handling
Materials	An assigned microscope Lens paper Immersion oil A methylene-blue-stained smear of *Candida albicans* (a yeast of medical importance) or other microorganism (the fixed, stained smear will be provided by the instructor)

Instructions

A. Important Parts of the Compound Microscope and Their Functions

1. Look at the microscope assigned to you and compare it with the photograph in figure 1.1. Refer to table 1.1 for a summary of the parts of the microscope and their functions. Notice that the working parts are set into a sturdy frame consisting of a *base* for support and an *arm* for carrying it. (*Note:* When lifting and carrying the microscope, always use *both hands;* one to grasp the arm firmly, the other to support the base (fig. 1.2). *Never* lift it by the part that holds the lenses.)

2. Observe that a flat platform, or *stage* as it is called, extends between the upper lens system and the lower set of devices for providing light. The stage has a hole in the center that permits light from below to pass upward into the lenses above. The object to be viewed is positioned on the stage over this opening so that it is brightly illuminated from below. The stage has clips for holding a glass slide in place (do not attempt to place your slide on the stage yet). Note the *stage adjustment knobs* at the side of the stage in figure 1.1, which are used to move the slide in vertical and horizontal directions on the stage. This type of stage is referred to as a *mechanical stage.*
3. A built-in *illuminator* at the base is the source of light. Light is directed upward through the *Abbe condenser.* The condenser contains lenses that collect and concentrate the light, directing it upward through any object on the stage. It also has a shutter, or *iris diaphragm,* which can be used to adjust the amount of light admitted. A lever (sometimes a rotating knob) is provided on the condenser for operating the diaphragm.

   The condenser can be lowered or raised by an adjustment knob. Lowering the condenser decreases the amount of light that reaches the object. This is usually a disadvantage in microbiological work. It is best to keep the condenser fully raised and to adjust light intensity with the iris diaphragm.
4. The *rheostat control knob* at the base is used for adjusting the intensity of light emitted by the bulb.
5. Above the stage, attached to the arm, a tube holds the magnifying lenses through which the object is viewed. The lower end of the tube is fitted with a *rotating nosepiece* holding three or four *objective lenses.* As the nosepiece is rotated with the knurled ring, any one of the objectives can be brought into position above the stage opening. The upper end of the tube holds the *ocular lens,* or eyepiece (a monocular scope has one; a binocular scope permits viewing with both eyes through two oculars). Some microscopes are set up with an ocular micrometer. This is a disk that is marked with a ruled scale and is placed in one of the ocular-lens eyepieces. The ocular micrometer has been precisely measured (calibrated) for that particular microscope at different magnifications by using a stage micrometer. The stage micrometer is a microscope slide with a calibrated scale marked on its surface. By measuring the distance between the lines of the ocular and stage micrometers at each magnification, conversion factors are obtained that are used to determine the size of objects viewed with the ocular micrometer. Ocular micrometers are especially useful in identifying protozoa and other animal parasite forms, for example, helminth eggs, as seen in figure 32.3. Your instructor may have a microscope demonstration of an ocular micrometer.
6. Depending on the brand of microscope used, either the rotating nosepiece or the stage can be raised or lowered by *coarse* and *fine adjustment* knobs. These are located either above or below the stage. On some microscopes they are mounted as two separate knobs; on others they may be placed in tandem (see fig. 1.1) with the smaller fine adjustment extending from the larger coarse wheel. Locate the coarse adjustment on your microscope and rotate it gently, noting the upward or downward movement of the nosepiece or stage. The coarse adjustment is used to bring the objective into position over any object on the stage, *while looking at it from the side* to avoid striking the object and thus damaging the expensive objective lens (fig. 1.3). The fine adjustment knob moves the tube to such a slight degree that movement cannot be observed from the side. It is used when one is viewing the object through the lenses to make the small adjustments necessary for a sharp, clear image.

   Turn the adjustment knobs *slowly* and *gently,* as you pay attention to the relative positions of the objective and object. Avoid bringing the objective *down* (or the stage *up*) with the fine adjustment while viewing, because even this slight motion may force the lens against the object. Bring the lens safely in place first with the coarse knob; then, while looking through the ocular, turn the fine knob to adjust the lens until you have a clear view of the subject.

   Rotating the fine adjustment too far in either direction may cause it to jam. If this should happen, *never attempt to force it;* call the instructor. To avoid jamming, gently locate the two extremes to which the fine knob can be turned, then bring it back to the middle of its span and keep it within one turn of this central position. With practice, you will learn how to use the coarse and fine adjustment knobs in tandem to avoid damaging your slide preparations.
7. The *total magnification* achieved with the microscope depends on the combination of the *ocular* and *objective lens* used. Look at the ocular lens on your microscope. On most microscopes, you will see that it is marked "10×," meaning that it magnifies 10 times.

   Now look at the three objective lenses on the nosepiece. The short one is the *low-power* objective. Its metal shaft bears a "10×" mark, indicating that it gives tenfold magnification. When an object is viewed with the 10× objective combined with the 10× ocular, it is magnified 10 times 10, or ×100. Among your three objectives, this short one has the largest lens but the least magnifying power.

Figure 1.1 The compound microscope and its parts. Courtesy of Olympus America, Inc. *©McGraw-Hill Education/James Redfearn, photographer*

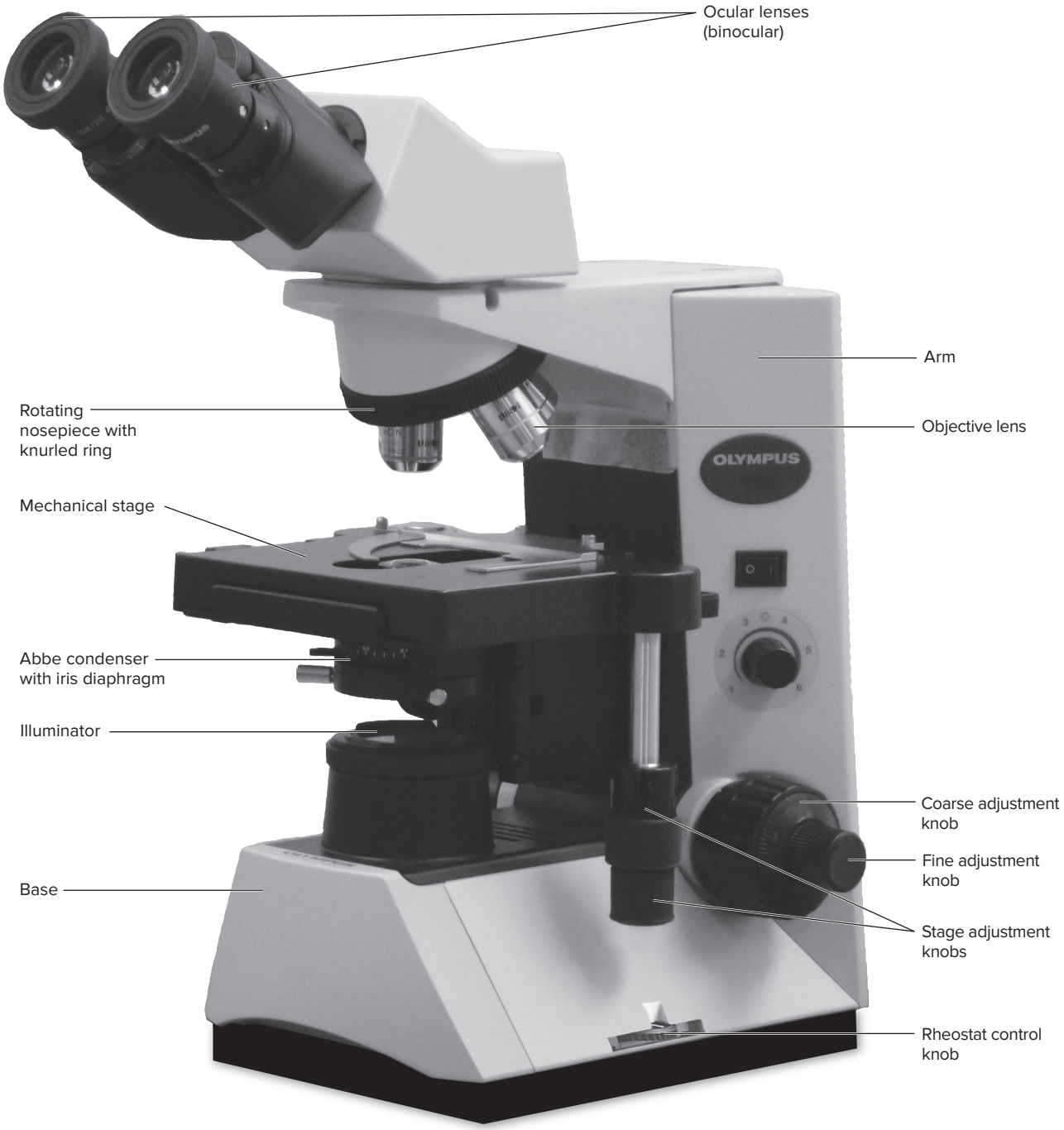


Table 1.1 The Parts of the Microscope and Their Functions

Microscope Part	*Function*	*Described in Instruction No.*
Base	Serves as support; holds the illuminator	A1
Arm	Provides support between the tube and base; used also for support when carrying the microscope	A1
Stage	A platform for holding slides; has a center hole to permit light to enter from below into the lenses; the mechanical stage allows easy movement of slides	A2
Stage adjustment knobs	Used to move the slide on a mechanical stage in vertical and horizontal directions	A2
Illuminator	Provides the source of light; located in the base	A3
Abbe condenser	Collects and concentrates light upward through the object on stage	A3
Iris diaphragm	Adjusts the amount of light entering the condenser	A3
Rheostat control knob	Controls the amount of light emitted by the bulb	A4
Tube	Holds the ocular lenses at the top end and the rotating nosepiece with objective lenses at the lower end	A5
Rotating nosepiece	Holds the objective lenses, which are rotated by means of the knurled ring	A5
Objective lenses	Lenses of different power to magnify the object on the stage; usually 10×, 40×, and 100× power	A5
Ocular lenses	Lenses through which the object on the stage is viewed; may be monocular or binocular; usually provides additional 5× or 10× magnification	A5
Coarse adjustment knob	Depending on the type of microscope, allows either the stage or nosepiece to be *carefully* raised or lowered, respectively, to provide quick focus	A6
Fine adjustment knob	Once the object is in focus with the coarse adjustment knob, provides "fine tuning" of focus	A6

Figure 1.2 Proper handling of a microscope. Both hands are used when carrying this delicate instrument. *©Josephine A. Morello*

Figure 1.3 When adjusting the microscope, the technologist observes the objective carefully to prevent breaking the slide and damaging the objective lens of the microscope. This dual-view microscope has two microscope heads combined for simultaneous viewing and is extremely useful for teaching purposes. *©Verna Morton*

The other two objectives look alike in length, but one is an intermediate objective, called the *high-power* (or *high-dry*) *objective.* It may or may not have a colored ring on it. What magnification number is stamped on it? _____ What is the total magnification to be obtained when it is used with the 10× ocular? _____

The third objective, which almost always has a colored ring, is called an *oil-immersion* objective. It has the smallest lens but gives the highest magnification of the three. (What is its magnifying number? _____ What total magnification will it provide together with the 10× ocular? _____) This objective is the most useful of the three for the microbiologist because its high magnification permits clear viewing of all but the smallest microorganisms (viruses require an electron microscope). As its name implies, this lens must be immersed in the drop of oil placed on the object to be viewed. The oil improves the *resolution* (the ability to distinguish detail) of the magnified image, providing a sharp image even though it is greatly enlarged. The function of the oil is to prevent any scattering of light rays passing through the object and to direct them straight upward through the lens.

Notice that the higher the magnification used, the more intense the light must be, but the amount of illumination needed is also determined by the density of the object. For example, more light is needed to view stained than unstained preparations.

8. The *focal length* of an objective is directly proportional to the diameter of its lens. You can see this by comparing your three objectives when each is positioned as close to the stage as the coarse adjustment permits. First place the low-power objective in vertical position and bring it down (or the stage up) with the coarse knob as far as it will go (gently!). When the object is in focus, the distance between the end of the objective, with its large lens, and the top of the cover glass is the focal length. Without moving the coarse adjustment, swing the high-power objective carefully into the vertical position, and note the much shorter focal length. Now, *with extreme caution,* bring the oil-immersion objective into place, making sure your microscope will permit this. If you think the lens will strike the stage or touch the condenser lens, *don't try it* until you have raised the nosepiece or lowered the stage (depending on your type of microscope) with the coarse adjustment. The focal length of the oil-immersion objective is between 1 and 2 mm, depending on the diameter of the lens it possesses (some are finer than others).

Never swing the oil-immersion objective into use position without checking to see that it will not make contact with the stage, the condenser, or the object being viewed. The oil lens alone is one of the most expensive and delicate parts of the microscope and must always be protected from scratching or other damage.

9. Take a piece of clean, soft *lens paper* and brush it lightly over the ocular and objective lenses and the top of the condenser. With subdued light coming through, look into the microscope. If you see specks of dust, rotate the ocular in its socket to see whether the dirt moves. If it does, it is on the ocular and should be wiped off more carefully. If you cannot solve the problem, call the instructor. *Never wipe the lenses with anything but clean, dry lens paper because solvents can damage the lenses.* Natural oil from eyelashes, mascara, or other eye makeup can soil the oculars badly and seriously interfere with microscopy. Eyeglasses may scratch or be scratched by the oculars. If they are available, protective eyecups placed on the oculars prevent these problems. If not, you must learn how to avoid soiling or damaging the ocular lens.
10. *If oculars or objectives must be removed from the microscope for any reason, only the instructor or other delegated person should remove them. Inexperienced hands can do irreparable damage to a precision instrument.*
11. Because students in other laboratory sections may also use your assigned microscope, *you should examine the microscope carefully at the beginning of each laboratory session. Report any new defects or damage to the instructor immediately.*

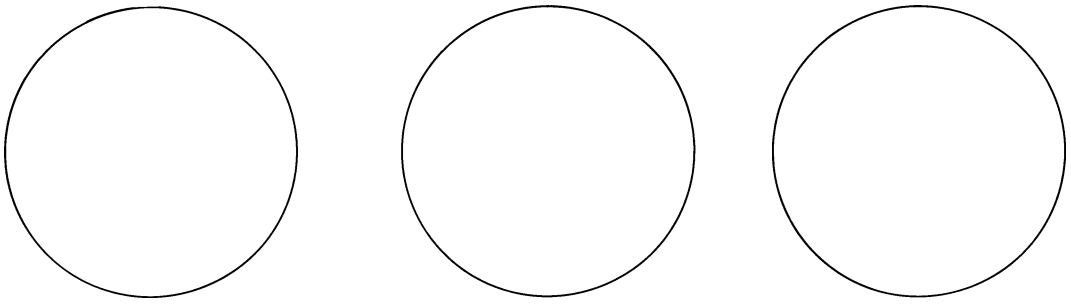
B. Microscopic Examination of a Slide Preparation

1. Now that you are familiar with the parts and mechanisms of the microscope, you are ready to learn how to focus and use it to study microorganisms. The stained smear provided for you is a preparation of a yeast (*Candida albicans*) that is large enough to be seen easily even with the low-power objective. With the higher objectives, you will see that it has some interesting structures of different sizes and shapes that can be readily located as you study the effect of increasing magnification. You are not expected to learn the morphology of the organism at this point (note that the instructor may substitute a different stained smear).
2. Place the stained slide securely on the mechanical stage fastened with the stage clips, making certain it cannot slip or move. Position it so that light coming up through the condenser passes through the center of the stained area.
3. Bring the low-power objective into vertical position and lower it (or raise the stage) as far as it will go with the coarse adjustment, observing from the side.
4. Look through the ocular. If you have a monocular scope, keep both eyes open (you will soon learn to ignore anything seen by the eye not looking into the scope). If you have a binocular scope, adjust the two oculars horizontally to the width between your eyes until you have a single, circular field of vision. Now bring the objective slowly upward or the stage downward with the coarse adjustment until you can see small, blue objects in the field. Make certain the condenser is fully raised, and adjust the light to comfortable brightness with the iris diaphragm.
5. Use the fine adjustment knob to get the image as sharp as possible. Now move the slide slowly around, up and down, back and forth. The low-power lens should give you an overview of the preparation and enable you to select an interesting area for closer observation at the next higher magnification. Record your observations in the circle labeled "Low-Power Objective" as described in step 9 on the next page.
6. When you have selected an area you wish to study further, swing the high-dry objective into place. If you are close to sharp focus, make your adjustments with the fine knob. If the slide is badly out of focus with the new objective in place, look at the body tube and adjust the lens close to, but not touching, the slide. Then, looking through the ocular, adjust the lens slowly, first with the coarse adjustment, then with the fine, until you have a sharp focus. Notice the difference in magnification of the structures you see with this objective as compared with the previous one. Record your observations as described in step 9.
7. Without moving the slide and changing the field you have now seen at two magnifications, wait for the instructor to demonstrate the use of the oil-immersion objective.
8. Move the high-dry lens a little to one side and place a drop of oil on the slide, directly over the stage opening. With your eyes on the oil-immersion objective, bring it carefully into position making certain it does not touch the stage or slide. Most microscopes are now *parfocal;* that is, the object remains in focus as you switch from one objective to another. In this case, the fine adjustment alone will bring the object into sharp focus. If not, while still looking at the objective, gently lower the nosepiece (or raise the stage) until the tip of the lens is immersed in the oil but is not in contact with the slide. Look through the ocular and very slowly focus upward with the fine adjustment. If you have trouble in finding the field or

getting a clear image, ask the instructor for help. When you have a sharp focus, observe the difference in magnification obtainable with this objective as compared with the other two. It is about $2\frac{1}{2}$ times greater than that provided by the high-power objective, and about 10 times more than that of the low-power lens.

9. Record your observations by drawing in each of the circles below several of the microbial structures you have seen, indicating their comparative size when viewed with each objective.
10. When you have finished your observations, lower the stage or raise the objective before removing the slide. Then remove the slide (taking care not to get oil on the high-dry lens). Gently clean the oil from the oil-immersion objective with a piece of dry lens paper.

Under each drawing, indicate the total magnification obtained by each objective combined with the ocular.



Low-Power Objective | High-Power Objective | Oil-Immersion Objective

Total Magnification: _____ _____ _____

C. Care and Handling of the Microscope

1. Always use both hands to carry the microscope—one holding the arm and one under the base (see fig. 1.2).
2. Before each use, examine the microscope carefully and report any unusual condition or damage.
3. Keep the oculars, objectives, and condenser lens clean. Use dry lens paper only.
4. At the end of each laboratory period in which the microscope is used, remove the slide from the stage, wipe away the oil on the oil-immersion objective, and place the low-power objective in vertical position.
5. Replace the dust cover, if available, and return the microscope to its box or assigned locker.

Table 1.2 suggests possible corrections to common problems encountered when using a microscope.

Table 1.2 Troubleshooting the Microscope

Problem	*Possible Corrections*
Insufficient light passing through ocular	Make certain the power cord is out of the way
	Raise the condenser
	Open the iris diaphragm
	Check the objective: is it locked in place?
Particles of dust or lint interfering with view of visual field	Wipe the ocular and the objective (*gently*) with clean lens paper
Moving particles in hazy visual field	Caused by bubbles in immersion oil; check the objective
	Make certain that the oil-immersion lens is in use, not the high-dry objective with oil on the slide
	Make certain the oil-immersion lens is in full contact with the oil.

Name _____ *Class* _____ *Date* _____

Questions

1. Why is focal length important when using the oil-immersion objective?

2. Describe the best way to adjust the amount of light entering your specimen.

3. If a 5× ocular lens were used with your microscope, what maximum total magnification could be achieved?

4. When should the coarse and fine adjustment knobs be used?

5. If you see moving particles in a hazy visual field, what steps can you take to obtain a clearer image?

6. What would you observe if you forgot to use oil with the oil-immersion lens?