Laboratory Safety: Introduction to the Microscope

EXERCISE 1

Exercise Pre-Test *Attempt to answer the following questions before starting this exercise. They will serve as a guide to important concepts. Recheck your answers after you complete this exercise and answer the laboratory report at the end of the exercise.*

EXERCISE 1

1. The term "eukaryotic" would refer to:
a. bacterial cells
b. human cells
c. cells with cell walls
d. cells with no organelles

2. The procedure used in microbiology to prevent contamination is:
a. antisepsis
b. asepsis
c. sanitation
d. disinfection

3. When using a standard compound microscope, the highest magnification is:
a. 100× total magnification
b. 400× total magnification
c. 1000× total magnification
d. 2000× total magnification

4. Which of the following should NOT be used with the microscope under high magnification?
a. coarse adjustment
b. iris diaphragm
c. stage
d. stage clip

5. A microscope focuses well under low power but not under higher magnifications. There is nothing wrong with the microscope. The most likely reason is:
a. the slide is upside down
b. the condenser is out of adjustment
c. too much oil was placed on the slide
d. the slide is too thick

OBJECTIVES

After completing this lab, you should be able to:

1 Explain why no eating, drinking, gum chewing, application of lipstick or other makeup, or smoking is permitted in the laboratory.

2 Locate the fire extinguishers and emergency exits in the laboratory.

3 Locate the fire blanket, eyewash station, and emergency shower.

4 Describe the procedure to follow when a chemical spill or other laboratory accident occurs. Identify the biohazard waste disposal container and the sharps container, and explain their correct usage.

5 Follow the proper procedures for starting and completing each laboratory session.

6 With your laboratory manual as a guide, focus your assigned microscope properly using the low-power, high-dry, and oil immersion objectives.

7 Be familiar with the most common mistakes students make in using the microscope.

8 Properly prepare a simple stain and recognize the differences between eukaryotic and prokaryotic cells.

Materials (provided by the student if required)

Laboratory manual assigned for each session

Lab coat with long sleeves and long enough to cover the lap when sitting

Permanent marking pen

Protective gloves

Safety eyewear

LABORATORY SAFETY AND PROCEDURES

One of the most important aspects of working in a microbiology laboratory is learning, and then following, established procedures for safety—*your safety*—as well as that of others. Besides awareness of fire and chemical hazards, you must understand that many microbes, if mishandled, are potentially hazardous to humans.

⚠ **SAFETY RULE:** NEVER PUT ANYTHING IN YOUR MOUTH WHILE IN THE LABORATORY, INCLUDING TOUCHING YOUR MOUTH WITH YOUR HANDS, TIPS OF PENS OR PENCILS, OR MOUTH PIPETTES.

⚠ **SAFETY RULE:** USE PROTECTIVE EYEWEAR AND GLOVES WHEN WORKING WITH CHEMICALS IN THE LABORATORY.

⚠ **SAFETY RULE:** DO NOT PLACE BACKPACKS, POCKETBOOKS, OR COATS ON THE LAB TABLES OR BENCHTOPS.

⚠ **SAFETY RULE:** AFTER EACH LAB IS FINISHED, WASH YOUR HANDS IMMEDIATELY WITH DISINFECTANT SOAP.

⚠ **SAFETY RULE:** INFORM YOUR INSTRUCTOR IF YOU ARE PREGNANT OR IMMUNOCOMPROMISED.

In this course, you will assume that *all* microbes are dangerous. One of the major ways that these microbes enter the body is by way of the mouth; thus, let us reiterate: no eating, drinking, gum chewing, or smoking in the lab, or anywhere else where hazardous microbes are routinely encountered.

Before and after each laboratory session, you will disinfect the tabletops and wash your hands immediately.

⚠ **SAFETY RULE:** REPORT ALL CUTS OR WOUNDS TO YOUR INSTRUCTOR.

Another way microbes can enter your body is through damaged skin. If you have any cuts or scrapes on your hand when entering the lab, or if you receive a wound that damages the skin while in the lab, make sure it is covered properly with an appropriate bandage or gloves before you perform any laboratory procedure.

In addition, you should observe the location(s) of various first aid and items of safety equipment around the laboratory. Listen carefully to your instructor's directions on how to use the fire blanket, eyewash station, deluge showers, and other safety equipment specifically used in your lab. Note the location of the fire extinguisher and exits. In the unlikely event of a laboratory fire, exit the building with your lab instructor. **DO NOT** leave your group for any reason until the Fire Marshal, Fire Department, or Security gives you permission to do so.

⚠ **SAFETY RULE:** REPORT ALL SPILLS AND ACCIDENTS TO YOUR INSTRUCTOR.

Inhalation is also a means by which dangerous substances enter the body.

⚠ **SAFETY RULE:** NEVER ENTER OR REENTER A LAB ROOM WHERE THERE IS SMOKE. TOXIC FUMES OFTEN DO NOT HAVE A STRONG ODOR.

During the remaining laboratory sessions, you will be working with many different kinds of microbes and chemicals. Although most of the materials with which you come into contact will not pose any real danger, we will assume that they are all potentially hazardous and must be handled appropriately. When you are required to use certain toxic chemicals, such as Kovac's reagent, your instructor will give you additional guidelines to follow.

⚠ **SAFETY RULE:** NEVER LEAVE THE LABORATORY WITH YOUR LAB COAT ON. PLACE YOUR COAT IN A POLY BAG BEFORE YOU LEAVE. REMOVE IT FROM THE LABORATORY ONLY TO WASH IT.

LABORATORY PROCEDURES

Microbiology laboratory procedures have long been established to keep bacteria, fungi, viruses, and other types of microbes under control while they are being studied. Such procedures have helped enhance the life span of many medical workers over the last 150 years. Cleanliness is an important aspect of this control as a clean, and disinfected, work area greatly reduces the number of all microorganisms, including those likely to cause disease. If the microbes are not present, they won't make you ill.

Aseptic technique (the word *aseptic* deriving from *sepsis* = infection or infectious material, *a* = without) is another extremely important aspect of laboratory procedure. Aseptic technique allows one to work with microbes without getting these potentially disease-causing organisms on the worker or work area, and, conversely,

getting extraneous microbes from the environment mixed in with those being studied. When such an event occurs, the worker, work area, or microbial sample becomes contaminated. A practical example of aseptic technique is the consistent use of handwashing by medical workers to prevent the spread of microbes from patient to patient.

To explain all of the aseptic technique procedures at this time would be somewhat overwhelming, for it is important to know both *why* you are performing a specific action and *how* to perform that action. This laboratory manual therefore addresses the preparation of the work area before actual microbiology activities take place as well as the procedures needed to clean up after the lab exercise is completed. As part of future lab exercises, other aseptic techniques will be introduced so that your knowledge of such procedures will gradually increase over the course of the semester.

The first and the last laboratory procedure you will perform every day is the cleaning of your work area with a disinfectant solution. Some disinfectants also provide the added benefit of serving as a cleaning agent. By wiping down your work area before the lab starts, you kill or remove microbes that may contaminate your work, as well as remove any residual dye, stain, or oil left by previous classes. And by repeating the same task at the end of class, you are leaving a clean, safe area for the next person.

SIMPLE STAIN TECHNIQUE

Stains provide better visualization of most objects seen under the microscope. Without such stains, cells are nearly transparent and extremely difficult to see. A *simple stain* is one in which a single colored dye or stain is used to tint the object. If a red dye is used as the stain, everything that accepts or absorbs the stain will appear red under the microscope. With certain types of cells, such as human cells, different components or organelles absorb different amounts of dye; thus, you will see various shades of the same color within the same cell. Because the nucleus of the human cell has a greater affinity for most dyes than the cytoplasm, the nucleus tends to show a darker shade of color than the rest of the cell.

MATERIALS PER STUDENT

Toothpick or cotton swab

Glass slides

China marking pencil or permanent marker

Staining tray

Bibulous paper or paper towel

Methylene blue stain or crystal violet stain

Prepared slides of cocci, bacilli, and spirilla

PROCEDURE

1. Prepare a cheek smear by *gently* scraping a toothpick or handle of a cotton swab along the inside of your cheek and then smearing this material on a marked section of a glass slide. *Discard* the toothpick or cotton swab in a disinfectant solution or as directed. If directed, repeat the procedure by scraping another toothpick or back of a cotton swab along your gumline.

   (*Note:* This is an example of aseptic technique. Any sample taken from an individual is considered potentially hazardous and cannot be thrown away in a regular garbage pail or wastepaper basket.) Figure 1.1 shows the preparation of a smear.

2. Allow the smear to *air-dry* (letting the moisture evaporate until the slide is dry) and then *heat fix* by holding the back of the slide against the port of the incinerator or in the flame of a bunsen burner. You may be directed to use a clothespin on the slide to prevent possible singed fingers. This procedure will adhere proteins, and thus cells, on the slide, as well as function to kill many cells. A drop of alcohol added to the smear will also effectively fix the cellular protein to the slide.

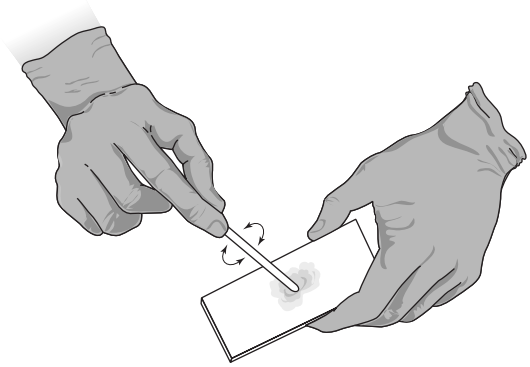


FIGURE 1.1 Preparation of a smear

3. Add enough *crystal violet* stain to cover the smear, and leave it on for approximately 5 seconds. If you are using *methylene blue,* leave the stain on for 1 minute. Your instructor will tell you which one to use. Figure 1.2 shows the stain being added to the slide on a staining tray.

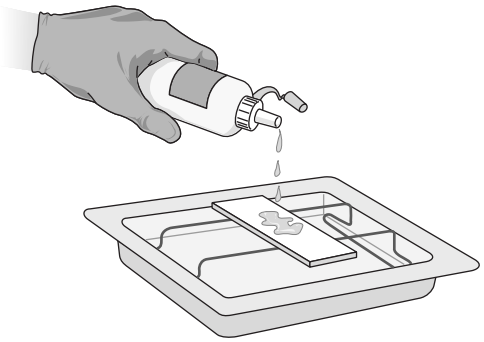


FIGURE 1.2 Adding stain to a slide

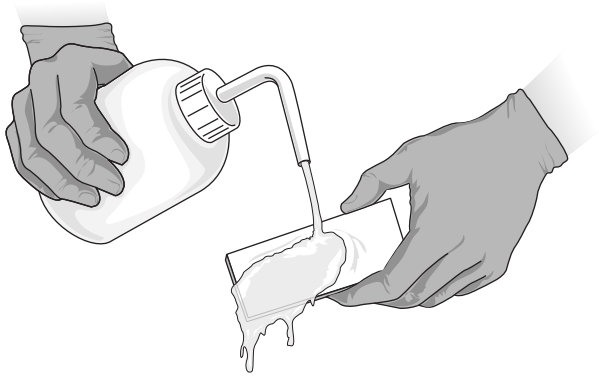


FIGURE 1.3 Rinsing off a slide

4. Rinse off with tap water from the sink or with the use of water bottles. Figure 1.3 shows the slide being rinsed off.
5. Blot dry with **bibulous paper** or a *paper towel*.
   (*Note:* Bibulous paper is specially prepared blotting paper with little or no extraneous paper fibers. This eliminates the sometimes annoying and confusing incidence of focusing on paper fibers under the microscope.) Figure 1.4 shows the slide being blotted dry.
6. Pour the residual stain in the staining tray into an appropriate discard container if directed to do so.

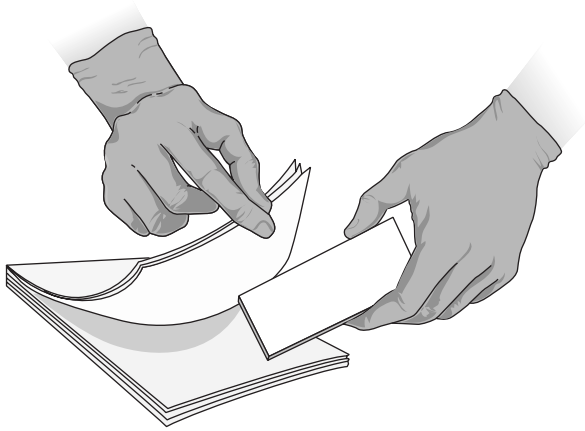


FIGURE 1.4 Drying a slide

SUMMARY

- Prepare cheek and gumline (optional) smear.
- Air-dry, heat fix.
- Perform simple stain.
- Rinse.
- Blot dry.
- Discard waste.

THE MICROSCOPE

A major component of this part of the course is staining and observing various types of microbes such as bacteria, fungi, and protozoans. (Viruses are too small to be seen with a conventional microscope.) Use of the microscope is an integral part of this study. These organisms, especially bacteria, are significantly smaller than any human or mammalian cells you may have seen in any Anatomy and Physiology or Biology course. Therefore, it is important that this device be used properly so that you can see the fine shapes, sizes, and structures of such small organisms. See Figure 1.5 for a chart showing the relative sizes of microscopic structures.

The type of microscope used in most courses is a bright field, binocular, compound microscope. It is a *bright field* because it projects bright light through the image on the slide; it is *binocular* because you can use both eyes to view the object; and it is *compound* because it uses a series of lenses to achieve magnifications of up to 1000 times. The following is a basic review and operating guide for using your microscope. Gaining familiarity with the microscope components and procedures for its use will certainly enhance your use of this instrument.

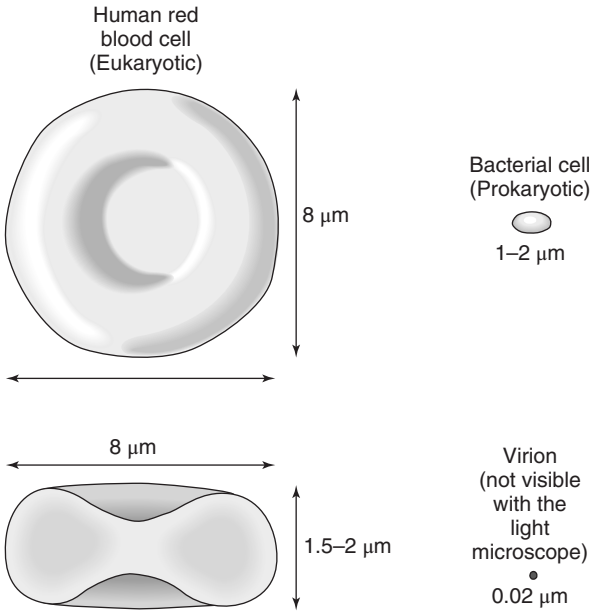


FIGURE 1.5 Comparison of microscopic structures

MICROSCOPE COMPONENTS (PLATE 1)

See Figure 1.6 and Plate 1 for different, labeled views of a compound microscope.

Ocular or eyepiece. The typical ocular has a 10X magnification; that is, it will magnify any object 10 times its size. If you are using a binocular microscope, you must make certain adjustments so that you can use both eyes to view the object on the slide. The ocular lenses must be adjusted to account for the distance between the eyes as well as for differences in focusing ability between the right and the left eye. Notice that the ocular lenses can be moved closer and further away from each other to adjust for the distance between the eyes, and they can also be focused independently of each other to adjust for the different focusing ability of each eye. Most binocular microscopes have one fixed focus ocular and one ocular that can be adjusted. (Some binocular microscopes allow both ocular lenses to be adjusted.)

With the adjustable lens set at the neutral ("0") position, focus the microscope at a higher magnification using only

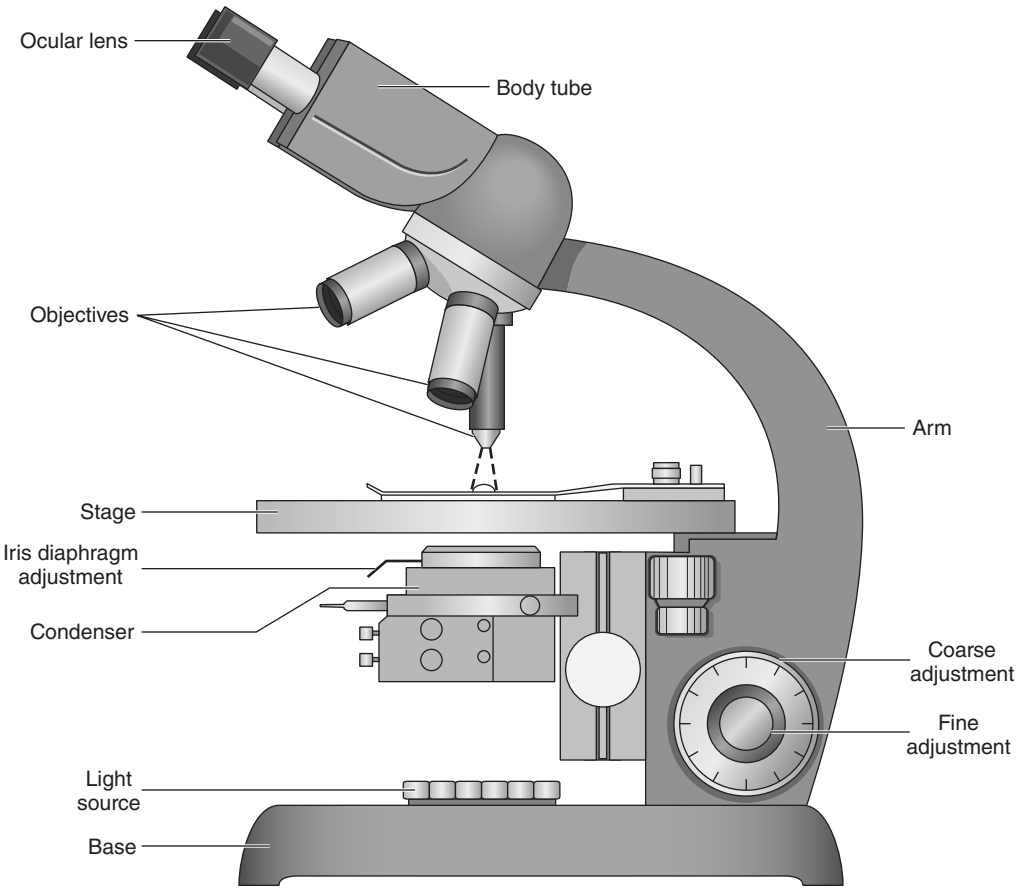


FIGURE 1.6
Compound microscope

the fixed focus lens. For example, if the fixed focus lens is the right lens, look through this lens with your right eye and adjust the microscope so that the target object is in focus. Once in focus, look through the adjustable ocular and determine whether the target object is still in focus. If in focus, both eyes are able to focus at the same point in space, and no further modifications are necessary. If the object looks out of focus with the adjustable lens set at "0," turn this lens clockwise or counterclockwise until the object is in focus. If you are near-sighted or far-sighted in one eye compared to the other eye, this procedure will adjust the microscope for your eyes, and no eyeglasses will be needed. If you have astigmatism, however, you will still have to use eyeglasses if you want to use both eyepieces.

Objectives. Three objective lenses are typically used in this course. The 10X objective or *low-power* lens, which will give a total magnification of 100X when used with the eyepiece (10 × 10), is used to initially focus the object on the slide. The 40X or *high-dry* objective lens (total magnification of 400X) will enlarge the object four times more than the 10X lens. It can also be used to select an interesting field of vision, or view, before changing to the 100X or *oil immersion* objective lens. Finally, the oil immersion objective lens (1000X total magnification) is used to view all the slides prepared in this class. Most microbes are so small that even at 1000 magnifications, they will be just visible. Notice that these lenses can easily be utilized by rotating the base or *nosepiece* where they attach to the body of the microscope.

Mechanical stage. The mechanical stage is where the slide is placed for viewing. Notice the stage clip that is used to hold the slide in place. Most microscopes have stages and stage clips that can be manipulated by turning two knobs located below the stage.

Coarse and fine adjustments. These knobs are usually located on both sides of the body at or below the level of the stage. The *coarse adjustment knob* is the larger of the two and is usually located closer to the body of the microscope. By turning it with the low power (10X) in place, you should readily see the stage move up and down unless the stage is at the highest position. ***The only time the coarse adjustment knob is used is when the low power lens is in place.*** If used with the higher power lenses, damage to the slide and the lens itself may occur. Most microscopes have a safety stop built into the coarse adjustment knob that prevents it from being raised too high. Once you reach this level, do not continue to turn the knob, for it may damage the microscope. Even with a safety stop, always view this adjustment of the lens and stage from the side to ensure that you do not damage any microscope components.

The *fine adjustment knob* is the smaller knob that is usually attached to the side of the coarse adjustment knob. By turning this knob, the stage will also move up and down but in much smaller increments. The movement is so minuscule that few students can see the stage move at all. One of these fine adjustment knobs may have a scale attached to it, which is useful to measure the thickness of cells under the microscope. Each notch on this scale usually measures an increment of only 2 μm, about one-fourth the diameter of a human red blood cell. This knob will be the only one used to focus the microscope when using the higher magnification.

Light. Most microscopes use the following components to adjust the light for optimum viewing.

Illuminator rheostat—Most microscopes make use of a **rheostat** to adjust the amount of electricity through the light bulb located beneath the stage. As the amount of electrical current increases, so does the illumination. Depending on the type of microscope used, your rheostat may be part of an on/off switch, or it may operate separately. Follow your instructor's directions in operating the rheostat.

Note: To prevent premature lamp burnout, the rheostat must be turned to its lowest position before turning the microscope on and off.

Condenser—The light is focused through the slide with this lens. Its ideal position is just below its highest position beneath the stage. To adjust this lens, locate the condenser focus knob under the stage and move the lens as directed, usually to its highest point.

Iris diaphragm—Once the rheostat and condensing lens are set, light passing through the slide can be regulated by simply adjusting the iris diaphragm located on the condenser itself. An adjustment knob or a lever is used to readily control the passage of light through the condensing lens. When using the microscope under low power (10X objective), adjust the opening of the diaphragm so that a minimum amount of light passes through the slide. As the magnification of the microscope is increased, you will have to increase the light transmitted through the slide by increasing the size of the opening of the iris diaphragm.

One of the most common problems students encounter with their microscopes is improper illumination. If you are having trouble seeing the object, the light adjustment should be one of the first things you should check.

PROCEDURE FOR USING THE MICROSCOPE

Step 1. Clean the lenses using *lens tissue* only. Start with the oculars and then the objectives. Clean the oil immersion objective last, so that any residual oil left from previous classes will not smear the oculars or other objectives.

Step 2. Set the microscope up as follows:

Have the 10X or low power objective in place.

Set the stage as high as it will go or lower the nosepiece to minimum working distance.

Set the rheostat, condensing lens, and iris diaphragm as directed.

Have the cheek cell slide, previously stained, or a prepared slide centered on the stage. (The light coming from the condensing lens acts as a spotlight to easily target the slide.)

Step 3. Look through the ocular and lower the stage. If you are looking at your own cheek smear, epithelial cells will eventually come into focus. If you are looking at a prepared slide, you will see extremely small structures. It may be advisable to focus in on the edge of the coverslip or a mark on the slide for the initial focusing. Once in focus, adjust the light and maneuver the slide so that the cells are in the center of the *field of vision*. (The circle of light you see through the ocular is the field of vision.)

Step 4. Without moving the stage, rotate the nosepiece until the high-dry (40X) objective is in place. Notice that this objective just barely misses the slide as it is rotated into place. This is why you must *use only the fine focus with the higher magnifications!* **USE OF THE COARSE ADJUSTMENT MAY DAMAGE THE SLIDE AND THE LENS!** You should notice that you can see the object, but it may be slightly out of focus. These microscopes are designed to be **parfocal**; that is, the lenses have been adjusted to focus at the same point in space. This means that if you get the object in focus under low power, it will be in focus (or nearly so) under the other magnifications. Once you use the fine adjustment to focus, you may have to adjust the light. The cells seen are now four times larger (400X vs. 100X), and the field of vision is now four times smaller. Because of this smaller field of vision, a cell on the periphery of the field at 100X magnification will not be seen at 400X magnification.

Step 5. Rotate the nosepiece once again until the oil immersion (100X) is about to lock into place. Place one to two drops of immersion oil on the slide in the location where the lens will rest, and then complete the rotation and lock the objective in place. The light from the condensing lens will guide you to the exact location for placement of the oil. The objective should now be touching the oil. Under this magnification, the oil increases the **resolution** of the microscope. That is, it will give a sharp, clear image. Focus with the fine focus knob and adjust the light using the iris diaphragm.

SUMMARY

- Clean lenses.
- Set up: low power (10X lens), light adjusted, stage up.
- Lower stage, coarse adjustment.
- 40X lens: rotate nose piece, adjust light, fine focus ***only***.
- 100X lens: rotate nose piece, add oil, adjust light, fine focus ***only***.

⚠ **REMEMBER: NEVER USE THE COARSE ADJUSTMENT WITH THE HIGH-DRY OR OIL IMMERSION OBJECTIVES.**

TROUBLESHOOTING

If you are having trouble getting the object in focus with the microscope under higher magnifications, consider the following:

1. Is the light adjusted properly? If not, review the steps in adjusting the light.
2. Is the slide upside down? If it is, you will get the object in focus under low power but not with the other objectives. *Hint:* Mark a part of the slide with a marking pen or pencil for a reference point. Prepared slides will have a label and coverslip, so this will not be a problem whenever these are used.
3. Was the object in focus under low power? Remember that these objectives are parfocal. If it is out of focus in low power, it will be out of focus with the others. If you are having trouble focusing the object under low power,

focus on a mark placed near the smear or on the edge of the coverslip.

4. Is the oil touching the lens? You will not get a high-resolution image with the oil immersion objective unless it is in contact with the oil.
5. Is the lens dirty? Use lens tissue to clean the lenses.

EUKARYOTIC VERSUS PROKARYOTIC CELLS (PLATES 2A, 2B)

One of the many methods of classifying organisms is to divide them into two major groups based on cellular structure. **Eukaryotic cells** (*eu* = true or real, *kary* = nuclear or chromosomal material), exemplified by human cells, have characteristics that include a membrane-covered nucleus with paired chromosomes and that tend to be relatively large. **Prokaryotic cells** (*pro* = before) have no nucleus, only one chromosome, and they are small compared to eukaryotic cells. Prokaryotic cells are bacteria. Human epithelial cells tend to be 30 to 40 μm in diameter, whereas most prokaryotic cells are only 1 to 2 μm wide. The most typical prokaryotic cell found on the surface of human cheek cells is a paired circular-shaped bacterium called a diplococcus (*diplo* = paired, *coccus* = berry or round). Even with 1000X magnification, the diplococci (*cocci* = plural of diplococcus) will just barely be visible. Figure 1.7 shows epithelial and diplococci together. The diplococci that will be observed under the microscope are most likely streptococci or chains of cocci when grown in the laboratory. In the mouth, they tend to be found as pairs.

Adjust the focus and light so that the bacterial cells are as clear as possible. Notice that if you rotate the fine adjustment knob so that the focus changes only 2 μm, these cocci will no longer be in focus. If you change the amount of light going through the slide by adjusting the iris diaphragm, notice that the cocci will be much more difficult to see. This is why you must be very precise in your operation of the microscope.

Observe these diplococci, see if you can also see three slightly different shapes of these cells. All three shapes are usually found in the mouth. If you have a good smear and a good stain, and if you focus the microscope properly, you will have a good chance of observing all three on your slide. (One type will look like perfectly round pairs of cells. Another type will look like two elongated letter *D*'s back to back, while the third pair will look like two kidney beans facing each other.)

If you took your cheek smear from the middle of your cheek, the most common bacterial (prokaryotic) cell type seen would probably be diplococci. If you took the sample closer to your throat, chains of cocci or streptococci may be observed. If the sample were from near your teeth, you would probably see several different cell types. Finally, if you did the scraping with increased pressure, you would likely see the nuclei of white blood cells in the mix. Food debris may also be observed if it has been a while since you brushed your teeth.

Draw some of the cheek cells and bacterial cells you observed. Use the box in Section E of the Laboratory Report. Be sure to note the size differences. You may wish to compare what was seen today with the bacterial cell types and arrangements seen in Figure 3.4 and in Plates 2A and 2B of the Photographic Atlas.

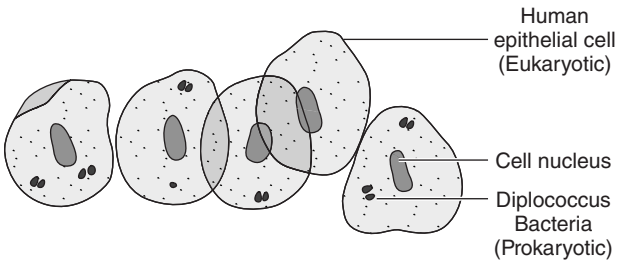


FIGURE 1.7 Human epithelial cells and diplococci

Now that you have observed your own personal array of bacterial cells, observe some of the different shapes (morphology) available in any provided prepared slides.

LABORATORY CLEANUP

An important part of this course is leaving your equipment and work area in proper condition for the next person to use.

MICROSCOPE CLEANUP

1. Return the objective lens to low power before you remove the slide.
2. Adjust the rheostat to dim the illuminator; then turn off the microscope lamp. As previously stated, this procedure increases the life of the bulb.
3. Clean the lenses of the microscope with *lens tissue only* in the same manner as before. Make sure that the low-power (10X) objective is pointing downward or, if instructed, between the objective lenses. Wipe all the oil off of the 100X objective and check the other objectives for oil.
4. Clean the stage if necessary.

5. Wrap the power cord around the microscope.
6. Cover the microscope if a cover is available, and store the microscope in its assigned place.

DISCARDS

Discard used and broken slides in the designated sharps container or in a container of disinfectant solution such as 10% bleach.

GENERAL CLEANUP

1. Wipe your work area down with disinfectant solution.
2. Return the stains, immersion oil bottles, staining trays, prepared slides, and all other equipment and materials to their proper locations.

SPEAKING OF SAFETY

The goal of every microbiology instructor is to create a safety consciousness in students that will continue to affect them in other laboratory courses, at home, and in the workplace years after the college laboratory experience is over.

A laboratory accident may seem an unlikely event to many students, yet year after year, hundreds of undergraduate laboratory accidents occur nationwide. Every precaution must be taken to assure a safe, enjoyable laboratory program.

⚠ REMEMBER THESE SAFETY RULES

1. No food or beverage is to be taken into a laboratory where accidental hand-to-mouth contamination or ingestion can occur. This is one of the most common ways in which dangerous microbes can enter the body.
2. Never place personal items such as backpacks or clothing on your laboratory table. Not only will they very likely get stained and dirty, but they also may become contaminated by microbes.
3. Wear appropriate dress: Use a lab coat, tie back long hair or use a hairnet, and put on protective gloves and eyewear when needed. Microbiological stains do one thing very well—they stain. Do not wear open shoes or apply makeup in the lab. It is better to get these stains on a lab coat than on your personal clothing. Long hair may get scorched in the Bunsen burner or pick up unwanted stains.
4. Know where the first aid kits, safety equipment, and exits are located in your laboratory. It is too late to study a floor plan when the lab is filled with fumes.
5. Follow your instructor's explicit instructions in the event that the laboratory must be evacuated. After evacuation, stay with your instructor. Never leave until you are permitted to do so.
6. Never put anything in your mouth while in the laboratory. No solutions should be pipetted by mouth. Again, this is one of the most common ways in which microbes can enter the body.
7. Never use a substance or chemical that is missing a label.
8. Always use the fume hood when instructed to do so. A chemical does not have to have an obnoxious odor to be toxic.
9. Notify the instructor in the event of a chemical spill or accident. Certain spilled chemicals will rapidly fill the lab with fumes.
10. If at all possible, do not share incinerators or Bunsen burners; this can lead to singed fingers.
11. Follow laboratory housekeeping rules such as washing down your tables with disinfectant before and after use. Allow the disinfectant to air-dry. For maximum effectiveness, do not towel dry the table. This will make for a clean and safe working environment.
12. Always wash your hands before leaving the laboratory room. (Are you detecting a pattern here?)
13. Replace your lab stool or chair under the table before you leave, and store your lab coat properly or place in a sealed plastic bag before you leave the lab. Make sure that all materials have been returned to their proper location.
14. Never work in the laboratory unsupervised.
15. Make sure that all gas jets are shut. If you smell gas, notify your instructor.

WORKING DEFINITIONS AND TERMS

Aseptic technique Procedures that prevent microbes from getting where they do not belong (contamination). This includes microbes from the environment contaminating your lab area as well as any microbes under study contaminating you or your work area.

Bibulous paper Specially prepared blotting paper that contains a minimum of loose paper fibers.

Eukaryotic Cells with a true nucleus and paired chromosomes.

Parfocal Feature of most microscopes that sets the focal point of all objective lenses at the same location in space.

Prokaryotic Cells with no true nucleus (membrane covered, paired chromosomes), with only one circular chromosome.

Resolution The ability to see a small object clearly under the microscope (technically, minimal distance at which two adjacent small objects can be distinguished as separate).

Rheostat Device that controls the amount of electrical current and thus the amount of light emanating from a bulb.

LABORATORY REPORT 1

NAME _____ DATE _____ SECTION _____

A. CRITICAL THINKING

1. Why are you not permitted to bring food into the laboratory?

2. Explain what can happen if you use the coarse adjustment with the oil immersion objective in place.

3. Why is the slide heated slightly once a smear has dried?

4. Name the two major different types of cells, and state at least two differences between them.

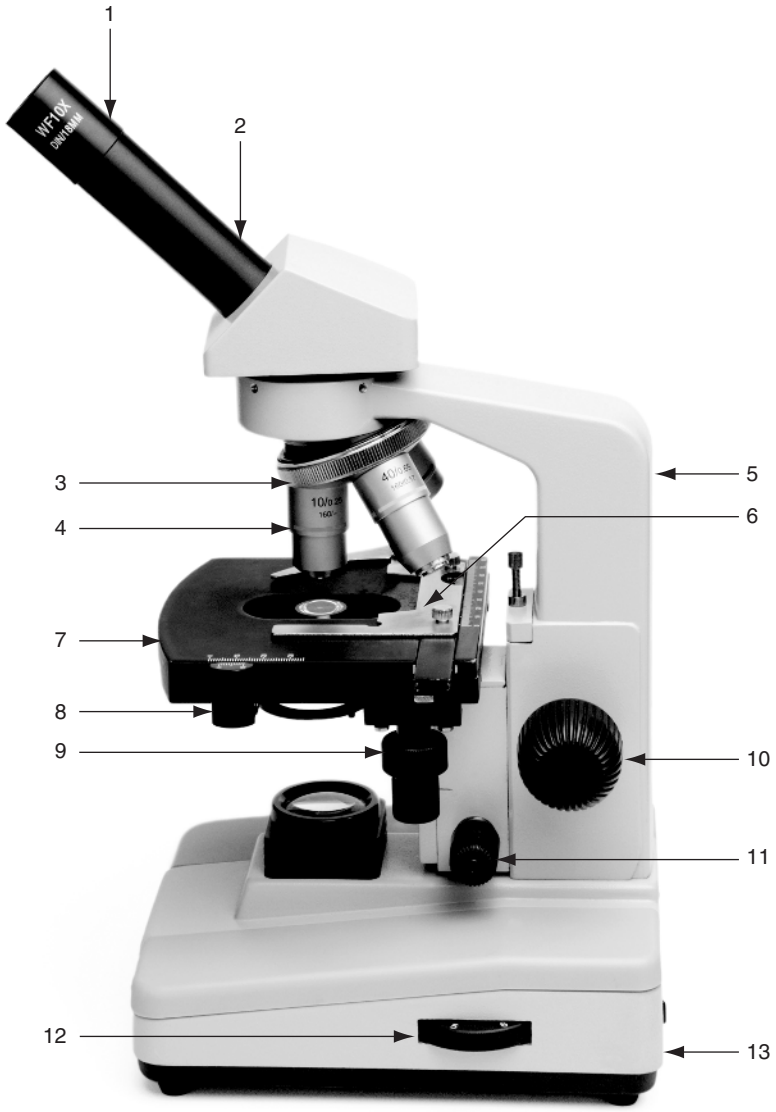
5. State five probable reasons why an object may be difficult to see under a properly working microscope.

B. MATCHING

a. coarse adjustment
b. fine adjustment
c. mechanical stage
d. nosepiece
e. iris diaphragm
f. condensing lens

1. ___ holds the slide in place while on the microscope
2. ___ used to initially focus the slide under low power
3. ___ holds the objectives in place
4. ___ used to focus the microscope under higher magnifications
5. ___ used to increase and decrease the light transmitted through the slide
6. ___ used to focus the light through the slide

C. LABEL THESE COMPONENTS OF THE MICROSCOPE



1. _____
2. _____
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8. _____
9. _____
10. _____
11. _____
12. _____
13. _____

D. MULTIPLE CHOICE

1. The major difference between the eukaryotic cell and prokaryotic cell is:
- **a.** nuclear material
- **b.** amount of cytoplasm
- **c.** presence of a cell wall
- **d.** one lacks a cell membrane

2. The size of a typical bacterial cell is approximately:
a. 2 µm **b.** 8 µm **c.** 30 µm **d.** 50 µm

3. A microscope slide focuses properly under low power but does not do so under oil immersion. Nothing is wrong with the microscope. The most probable reason is:
- **a.** the light is not adjusted properly
- **b.** the slide was not heat-fixed properly
- **c.** the slide was placed on the microscope upside down
- **d.** the coarse adjustment was not used under low power

4. Which of the following is a probable source of contamination for the microbiology student?
- **a.** eating in the laboratory
- **b.** placing personal objects on the laboratory table
- **c.** chewing gum in the laboratory
- **d.** all of these

5. The most common shape of bacteria seen in a typical cheek smear is:
a. streptococci **b.** spirilla **c.** diplococci **d.** single rod

6. The function of a condenser on a light microscope:
- **a.** adjusts the intensity of light going through the slide
- **b.** concentrates the light onto the slide
- **c.** magnifies the object before it reaches the ocular
- **d.** controls the electricity that goes through the light source

7. A parfocal microscope:
- **a.** uses two eyepieces
- **b.** allows the user to view an object in three dimensions
- **c.** allows you to switch objectives without making major changes in focusing
- **d.** uses more than one lens to achieve final magnification

8. Total magnification of a light microscope is achieved when:
- **a.** magnification power of the condenser × magnifying power of the ocular
- **b.** magnification power of the condenser × magnifying power of the objective lens
- **c.** total magnification power of all objectives added together
- **d.** none of these

E. LABORATORY REPORT

A Draw the results of your cheek smear. Be sure to show the size difference between eukaryotic and prokaryotic cells.

B Draw the results of your gumline smear. Again, show the size difference between the human eukaryotic and prokaryotic cells.