Laboratory Skills

TOPIC 9

What do You Think?

Laboratory Procedures

When you are looking at cells with a microscope always start focusing with the high power objective lens, then if the cells appear too large, switch to a lower power.

You shouldn't eat food or drink soda in a classroom laboratory unless you make sure you wash your hands first.

Paper chromatography is used to separate mixtures of molecules that are of different sizes.

Gel electrophoresis is used to cut DNA into smaller fragments.

TOPIC 9

Laboratory Skills

Vocabulary

balance
chromatography
compound light microscope
dichotomous key
dissection
electronic balance
electrophoresis
graduated cylinder
indicator
magnification
mass
metric ruler
microscope
stain
stereoscope
triple-beam balance
volume

Topic Overview

Biologists are always trying to add to our understanding of the world in which we live. To do this, they make observations and do experiments. To help them collect data from these observations and experiments, they rely on a variety of tools and techniques. These tools, like microscopes, extend our senses, allowing us to collect data that would otherwise be unavailable. Scientists have also developed specific techniques that give us valuable information about the cells, molecules, and processes of living organisms.

Tools for Measurement

During laboratory investigations, you are often required to make measurements of length, mass, and volume. You need to know the proper pieces of equipment to select and the appropriate procedures and units to use.

Measuring Length Typically a **metric ruler** is used to determine the length of an object. To measure length, use either centimeters (cm) or millimeters (mm). You should know how to convert millimeters to centimeters and vice versa.

The metric ruler shown in Figure 9-1 is calibrated—or scaled—in centimeters (cm). The lines indicated by the numbers 1, 2, 3, and so on each represent a distance of 1 centimeter. The smaller divisions each equal 1 millimeter (10 mm = 1 cm). A line equal to 5 cm is shown above the ruler in Figure 9-1.

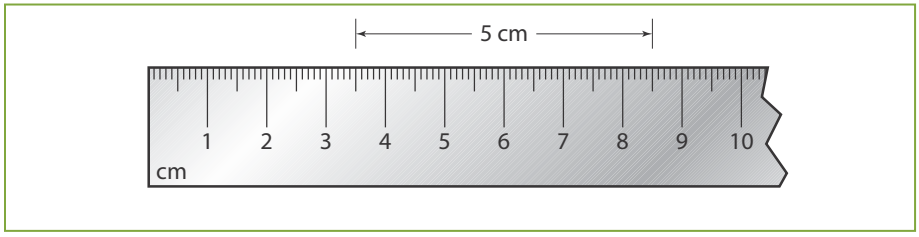


Figure 9-1. Metric ruler: The tool used for measuring length in centimeters and millimeters (not shown to scale)

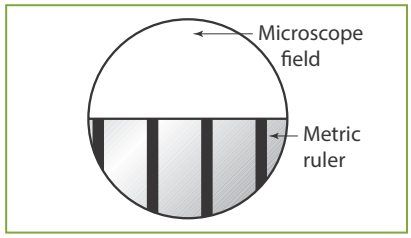


Figure 9-2. Microscope field with metric ruler (not shown to scale)

Micrometers (μm), are very tiny units that are used to measure objects through the microscope. One thousand micrometers equal one millimeter.

Figure 9-2 shows a metric ruler as seen under the low-power objective of a microscope. The distance across the field of view is approximately 3.2 millimeters, or 3,200 micrometers (since 1.0 mm = 1,000 μm).

If the cells observed on the slide in Figure 9-3 are being viewed through the same low-power objective, the field of view is still 3.2 millimeters. The approximate length of each cell is therefore about 1 millimeter or 1,000 micrometers, since about 3 cells fit across the diameter (widest part) of the field.

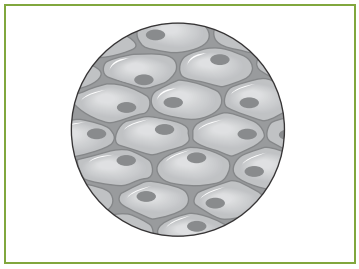


Figure 9-3. Microscope field with cells

Measuring Volume A **graduated cylinder** is often used to measure a liquid's **volume**, or the space it occupies. Liters and milliliters are typically used to indicate volume in the metric system, while quarts and ounces are used in the English system. Graduated cylinders are calibrated in milliliters (mL).

Water and many other fluids form a meniscus (curving surface) when placed in the narrow tube of a graduated cylinder. To correctly read the volume of the liquid, place the cylinder on a flat surface. Then read from the bottom of the curved meniscus at eye level. The volume of liquid in the graduated cylinder in Figure 9-4 is 13 mL.

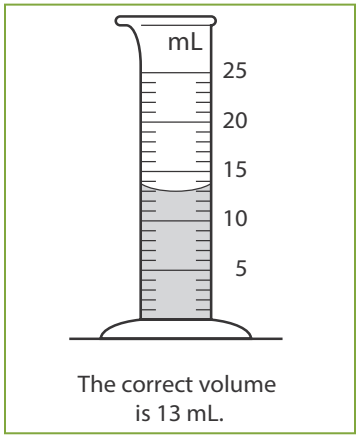


Figure 9-4. Graduated cylinder: You can find the volume of a liquid using a graduated cylinder.

Measuring Temperature In the biology laboratory, temperature is often measured in degrees Celsius. The freezing point of water is 0°C; the boiling point is 100°C. Human body temperature is 37°C, which is the temperature indicated on the thermometer in Figure 9-5.

Measuring Mass In the biology laboratory, **mass**—the quantity of matter in something—is often measured with a **balance**, which is a tool that works by comparing an object of unknown mass with an object of known mass. The triple-beam balance or an electronic balance is typically found in a high school laboratory.

A **triple-beam balance** (Figure 9-6) has a single pan and three bars (beams) that are calibrated in grams. One beam, the 500-gram beam, is divided into five 100-gram units. Another beam is divided into ten units of 10 grams totaling 100 grams. The front beam is divided into 10 major units of 1 gram each. Each of these divisions is further divided into 0.1-gram units.

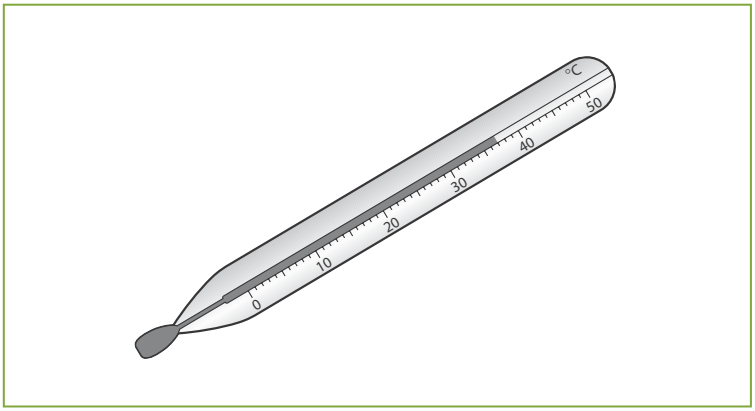


Figure 9-5. Thermometer: This Celsius thermometer shows human body temperature.

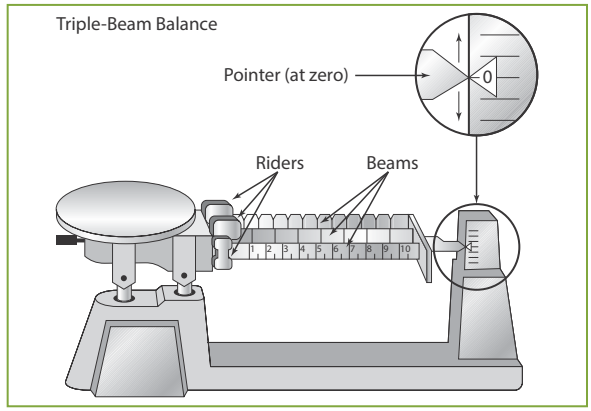


Figure 9-6. Triple-beam balance

Figure 9-7. **Electronic balance**

Before using a balance, make sure that the pan is empty and that the pointer and all of the riders (devices that are moved along the beams) are on zero. To determine the mass of an object, it is first placed on the pan. Then, starting with the 500-gram beam, the masses on the beams are adjusted until the pointer is again pointing to zero. The mass of the object is equal to the sum of the readings on the three beams.

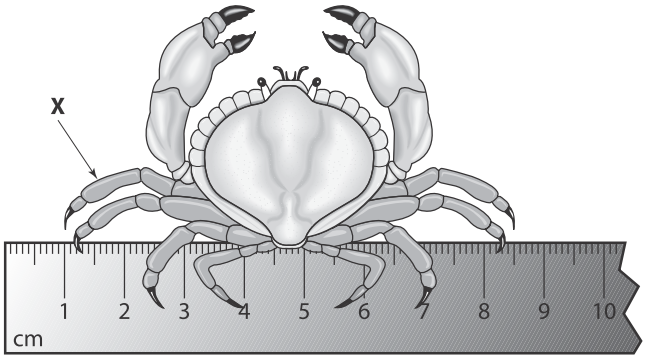
An **electronic balance** measures mass automatically. To use an electronic balance, first turn it on and wait until it shows a zero mass. (This may require using the re-zero button shown on Figure 9-7.) Place the object with the unknown mass on the pan. Read the mass.

Never place a substance directly on a balance pan. Instead, protect the balance with a weighing paper or dish. With a triple-beam balance, first find the mass of the weighing paper. Then find the mass of the substance AND the weighing paper. Subtract the mass of the weighing paper from the total mass of the paper plus the substance. The remainder is the mass of the substance.

For an electronic balance, put the weighing paper on the balance and then use the re-zero button to set the balance to zero. Next, put the substance on the paper and read the mass. The re-zero button automatically subtracts the mass of the weighing paper from the total. The reading on the balance is the actual mass of the substance.

Review Questions — Set 9.1

1. The crab shown in the illustration below has four pairs of walking legs and one pair of pincer legs. The crab is shown in its normal walking position.



In this position, what is the distance between the ends of the front pair of walking legs? (One of the front pair of walking legs is identified with an "X" in the illustration.)

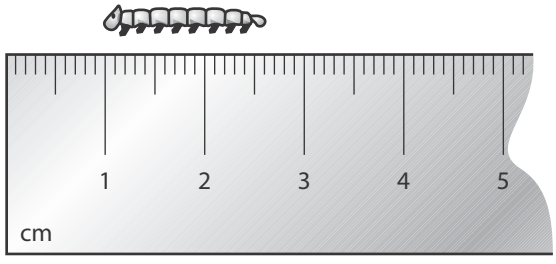
(1) 8.5 cm
(2) 85 cm
(3) 7.5 cm
(4) 75 cm

2. Identify which piece of laboratory equipment you would use to accurately measure 10 grams of glucose. [1]

3. Which piece of laboratory equipment would be used to most accurately measure the volume of a liquid?

(1) beaker
(2) balance
(3) test tube
(4) graduated cylinder

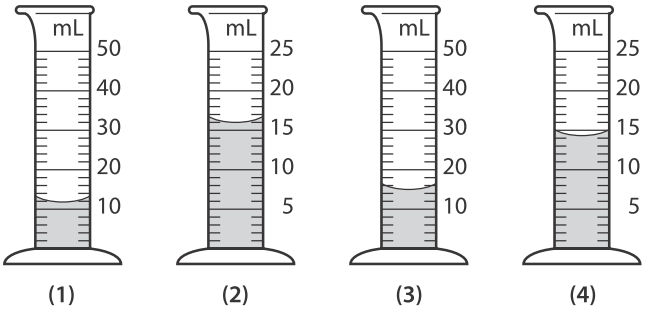
4. A student measured a larva using a metric ruler, as represented in the diagram below.



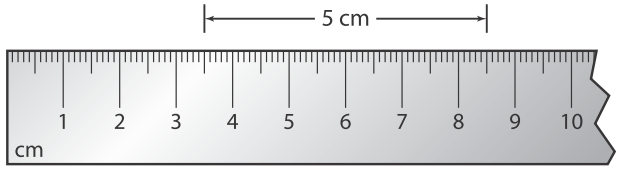
What is the length of the larva?

(1) 26 cm (2) 26 mm (3) 16 cm (4) 16 mm

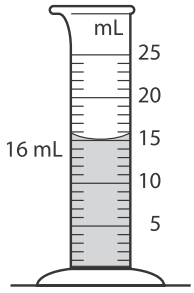
5. Which of the graduated cylinders below contains a volume of liquid closest to 15 mL?



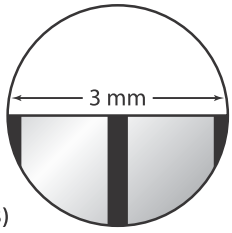
6. Which diagram below shows a correct measurement?



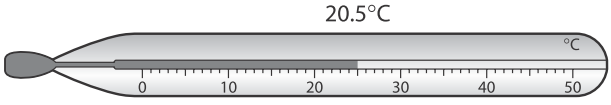
(1)



(2)

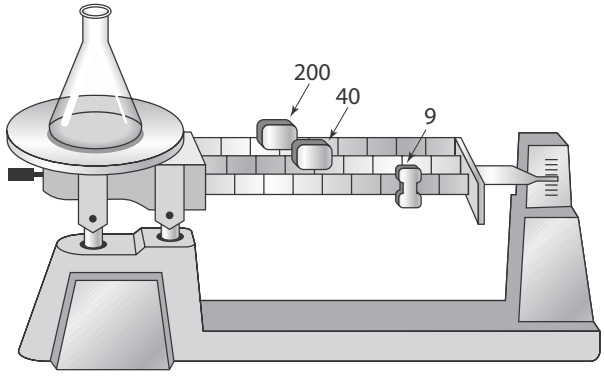


(3)



(4)

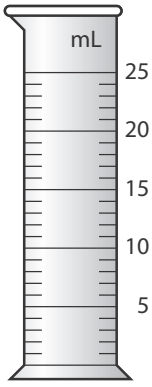
7. The diagram below shows a triple-beam balance with a mass on the pan.



With the riders in the positions indicated, what is the mass of the object on the pan of the balance?

(1) 9 grams
(2) 200 grams
(3) 249 grams
(4) 942 grams

8. Draw a meniscus to represent a water level of 6 mL on the diagram below of a graduated cylinder. [1]



Microscope Skills

The **microscope** is a tool that uses a lens or a combination of lenses to make an object easier to see. It allows for the examination of objects too small to be seen with the unaided eye. Microscopes also permit the close observation of fine details. For example, without a microscope you can see the legs and wings of a fly. With a microscope you can also see the hairs covering the fly's body, the pads and clawlike structures on its feet, and the framework of its wings. This is possible for two reasons. A microscope magnifies the specimen and also allows you to distinguish between objects that are close together. **Magnification** is the ability of a microscope to make an object appear larger.

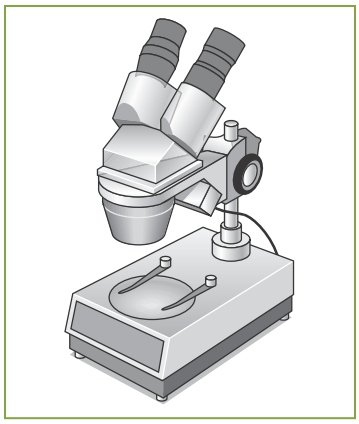


Figure 9-8. **A stereoscope**

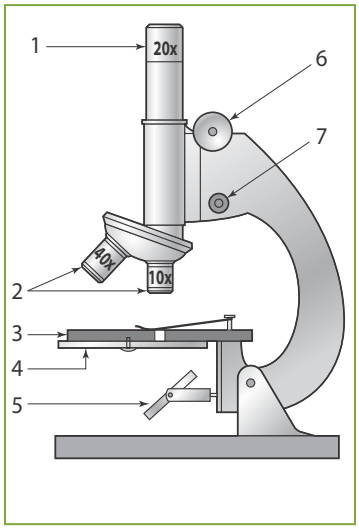


Figure 9-9. **A compound microscope:** Each part is labeled with a number. The names and functions of the parts are given in Table 9-1.

Types of Microscopes

There are many different types of microscopes. The two most commonly found in a high school laboratory are the compound light microscope and the stereoscope. The primary difference between the two is that with a compound light microscope, light must pass through or reflect off the specimen being examined.

Stereoscopes With a stereoscope (Figure 9-8), light is reflected off the specimen. A **stereoscope**, sometimes called a dissecting microscope, has two ocular eyepiece lenses, one for each eye, and one or more objectives (the other lenses of the microscope). The amount of magnification is low, but the image is three-dimensional and is not reversed as it would be with a compound microscope. Stereoscopes are often used to observe parts of specimens such as insects, worms, or flowers.

Compound Microscopes The typical **compound light microscope** has one ocular lens, at least one objective lens, and a light source. Light passes through the object being examined, through the objective lens, and then through the eyepiece.

The image you see is magnified by both lenses—the ocular lens and the objective lens. The total magnification is calculated by multiplying the magnification of the ocular by the magnification of the objective. For example, if you use a microscope that has a 10x eyepiece and a 40x objective, the magnification of a specimen would be 400x.

Eyepiece × Objective = Total Magnification

10x × 40x = 400x

Table 9-1. Names and Functions of Parts of a Compound Microscope

1 Eyepiece or Ocular Lens	• lens nearest the eye and used to "look through" • usually magnifies 10x
2 Objective Lenses	• lenses located closest to specimen • usually 2 or 3 • commonly magnify at 4x, 10x, and 40x
3 Stage	• flat surface (platform) on which the slide is placed • stage clips hold the slide in place
4 Diaphragm	• located under the stage • controls the amount of light passing up through the specimen
5 Light Source	• might be a mirror or a light bulb • provides light that passes up through the specimen and makes it visible
6 Coarse Adjustment	• used to focus only under low power (up to 100x) • never used when the high-power objective is in place for viewing • usually the larger knob; causes a large amount of movement of the lenses
7 Fine Adjustment	• the only focus you should use with high power • used to sharpen the image under low power • also used to see different layers of a specimen • usually the smaller of the focus knobs; causes a small amount of movement of the lenses

Microscope lenses may get dirty from contact with fingers, specimens, stains, and so on. Do not use paper towels or your shirt to clean them! Only use lens paper to clean the lenses of a microscope. Lens paper will not scratch the soft glass of the lens.

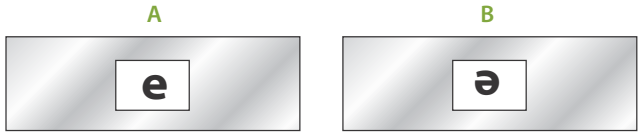


Figure 9-10. Microscope view: The letter "e" as seen on a slide (A) and as seen through the microscope (B). (Only the change in position of the letter is shown, not magnification.)

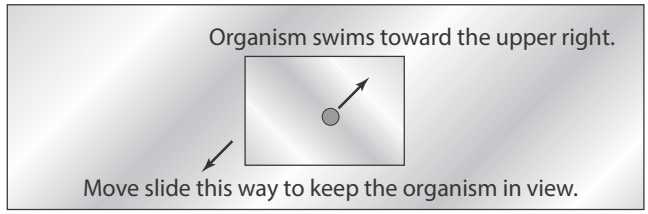


Figure 9-11. Moving the slide to follow a moving object

Techniques for Using Microscopes

Due to the action of microscope lenses, there are a number of things you need to remember when viewing objects through a compound light microscope.

- The image will be upside-down and backwards, as shown in Figure 9-10.
- You must move the slide in the direction that is opposite the way the organism appears to be moving. In other words, if the organism appears headed toward the upper right side of your field of view, you must move the slide down and to the left to keep it in view. See Figure 9-11.
- The field becomes darker as you increase the magnification. You will need to increase the amount of light passing through the specimen as you go from low power to high power. The diaphragm, located under the stage, can be used to do this.
- Since the field becomes smaller under high power, center the object you are viewing before switching to a higher power. Otherwise the object may be outside of the field of view.

Focusing When observing specimens through the compound light microscope, first use the low-power objective. Do this even if higher magnification is needed to make your observations.

1. First, place the slide on the stage of your microscope. Position the slide so that the specimen is over the opening in the stage. Anchor the slide with the stage clips.
2. Move the coarse adjustment so that the low-power objective is as close to the slide as you can get it without touching the slide. Some microscopes have a built-in "stop" that prevents you from getting the objective lens too close to the slide. You should look at the objective and the slide while doing this. Never lower the objective while looking through the eyepiece.
3. Look through the eyepiece with both eyes open and turn the coarse adjustment so that the low-power objective and slide move apart. The specimen should come into view.
4. Next, turn the fine adjustment to bring the specimen into sharp focus.
5. To focus the specimen under higher magnification after locating it under low power, move the slide so that what you are interested in seeing is located in the center of your field of view. Remember that as you increase the magnification, the object appears larger, but you see less of its edge. The field of view becomes smaller when you

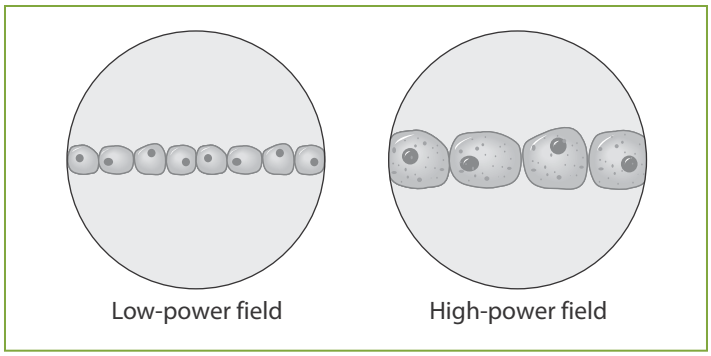


Figure 9-12. Specimen under low and high power: Note that the high power field is narrower (only 4 cells can be seen), but the cells appear larger, and more detail is visible.

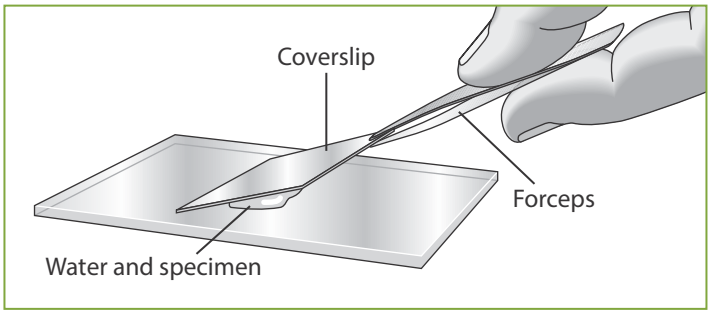


Figure 9-13. Preparing a wet-mount slide

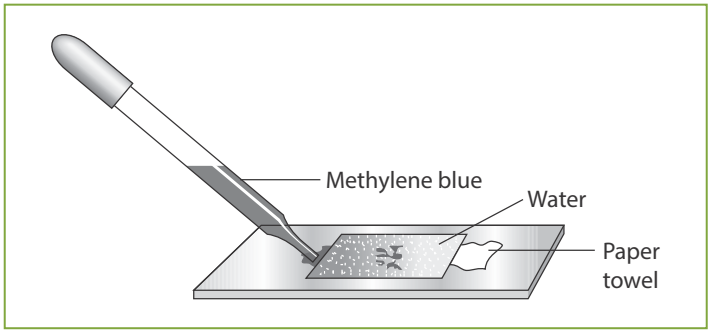


Figure 9-14. Staining a wet-mount slide

switch from low power to a higher power. See Figure 9-12.

6. Watch from the side of the microscope and slowly turn the high-power objective into place. Be sure that the high-power objective is not going to touch the slide. High-power objectives are longer than low-power objectives and can easily hit the slide—be careful.
7. If the objective is not going to hit the slide, click it into position. As you look through the eyepiece, the specimen should be visible. Use the fine adjustment to sharpen the focus. Remember never to use the coarse adjustment when using the high-power objectives. You could damage the microscope lens and break the slide.

Preparing Wet-Mount Slides Only specimens that are small and thin can be seen through a compound light microscope. However, thin slices may quickly dry and shrivel. To avoid this, a temporary wet-mount slide can be prepared by using the following steps:

1. Using a pipette (eye dropper), add a small drop of water to the center of a clean, glass slide.
2. Place the object to be viewed in the water. (It should be lying flat rather than folded over.)
3. Use forceps to position a coverslip, as shown in Figure 9-13. Using forceps will keep you from getting fingerprints on the coverslip. Fingerprints could interfere with your ability to view the image clearly.
4. Lower the coverslip slowly. This technique will prevent the formation of air bubbles under the coverslip.

Staining Specimens When you examine cells and cell parts through a microscope, they often appear to be transparent. You need to adjust the light and the focus so that you can see differences in thickness and density. Although adjusting the amount of light passing through the specimen may help, stains are often used to create greater contrast. Different types of cells and cell parts vary in their ability to soak up various stains. For example, certain cell parts turn darker in the presence of iodine stain. Other parts do not become darker.

To add a stain, such as methylene blue, to a wet-mount slide, place a drop of the stain beside one edge of the coverslip. Next touch a small piece of paper towel to the opposite edge of the coverslip. The towel absorbs water and draws the stain across the slide under the coverslip. This technique allows you to keep the slide on the stage of the microscope. You do not need to prepare a new slide. See Figure 9-14.

Identifying and Comparing Cell Parts It is important to remember that cells have specific structures that perform specific jobs. Many of these structures in Figures 9-15 and 9-16 are visible through a compound light microscope. Some of the parts you can either expect to see or see evidence of are the following:

- **Nucleus**—usually observed as a rounded, dense, dark-staining structure. It can be located anywhere in the cell, not just in the middle.
- **Cytoplasm**—typically fills the cell. It appears to be clear in some cells and very grainy in others. Cell organelles, which may or may not be visible, are suspended in the cytoplasm.
- **Cell membrane**—found surrounding the cytoplasm. It is the outer boundary of animal cells and is located between the cell wall and the cytoplasm in plants and some other organisms.
- **Cell wall**—The nonliving cell wall on the outside of the cell membrane in plant cells is a supportive structure. Many bacteria form a different type of protective cell wall.
- **Chloroplasts**—green, oval structures found in the cytoplasm of some plant cells and photosynthetic one-celled organisms.
- **Vacuoles**—often seen as clear areas in the cytoplasm. Plant cells contain very large fluid-filled vacuoles that occupy much of the inside of the cell. Some single-celled organisms may contain specialized vacuoles for digestion and for regulating water balance.
- **Chromosomes**—most easily observed in cells undergoing mitosis or meiosis as in Figure 9-17. They are usually dark-staining and threadlike.

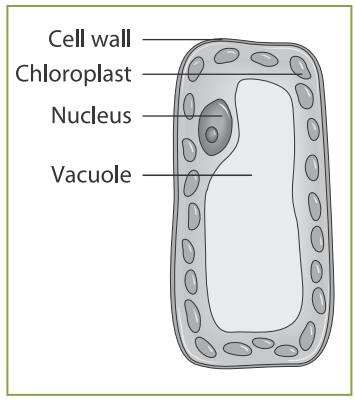


Figure 9-15. Typical plant cell: Structures that can be seen through a microscope

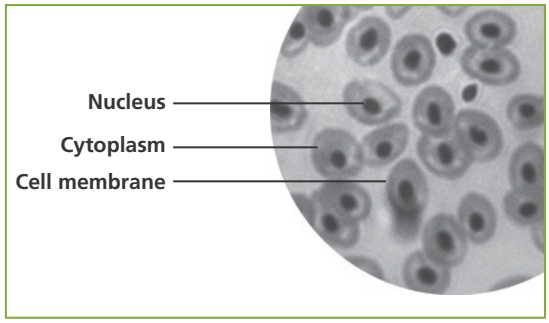


Figure 9-16. Animal cells: As seen through a compound light microscope

Additional Laboratory Techniques

There are many techniques that are useful in the biology laboratory. Some of the most common are electrophoresis, chromatography, and the application of stains and indicators. Dichotomous keys are especially useful for field research.

Electrophoresis Gel **electrophoresis** is a very powerful tool and is widely used by scientists in many disciplines—not just biologists. It allows scientists to separate mixtures of large molecules according to size. DNA and proteins are the two types of molecules most often separated by gel electrophoresis.

When setting up a protein gel, a sample of biological material containing proteins is prepared by breaking open the cells in order to release the proteins. Next, the proteins are treated with both chemicals and heat. One of the chemicals used coats the protein molecules and gives them a negative charge. Then, very small amounts of the prepared sample are placed in wells at the top of a special gel positioned in a gel electrophoresis apparatus. (The wells are similar to the holes you would get by pressing the teeth of a comb part way into a block of gelatin dessert.) The gel is placed between two electrodes that are connected to a power supply. This causes one end of the gel to take on a positive charge and the other to take on a negative charge.

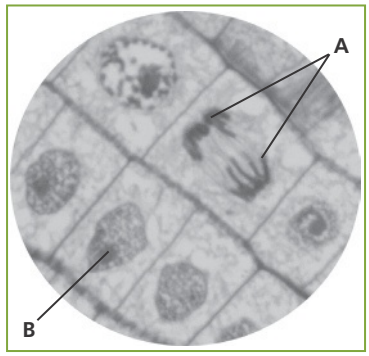


Figure 9-17. Plant cells undergoing mitotic cell division: A indicates chromosomes, and B indicates a nucleus before the cell undergoes mitosis.

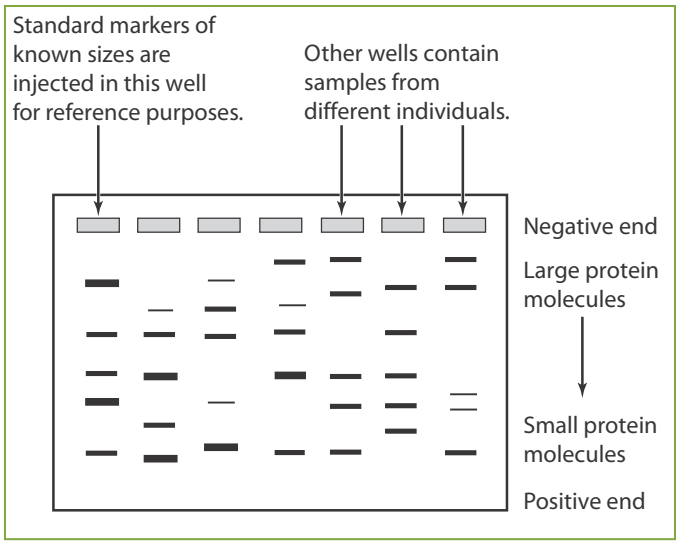


Figure 9-18. Protein gel electrophoresis: Protein gels are typically run vertically, with the gel block standing on end.

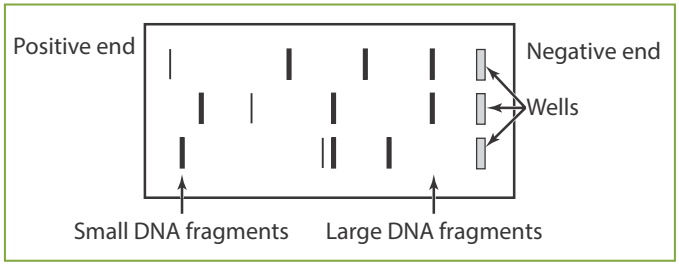


Figure 9-19. DNA gel electrophoresis: DNA gels are typically run horizontally, with the gel block lying flat.

Positively charged molecules in the sample move toward the negative electrode, while negatively charged protein molecules move toward the positive electrode. The type of gel used in protein electrophoresis is made up of long molecules that form a tangled mesh. Smaller molecules are able to work their way through the gel more quickly than larger molecules. Therefore, molecules are separated by both their size and electrical charge. See Figure 9-18.

DNA gel electrophoresis is a little different. The analysis of an individual's DNA begins with the use of special enzymes to cut the DNA at specific points in the sequence of bases. This produces fragments of DNA that are of different lengths. These pieces of DNA will vary in size and number from one individual to another due to the uniqueness of each genetic code.

Next, small amounts of the DNA samples are placed in wells located on one side of a semisolid gel. See Figure 9-19. Typically the DNA gel is made of agarose—the same gelatine-like substance used to culture bacteria but without the nutrients. The gel is located between two electrodes that are connected to a power supply. This causes one end of the gel to take on a positive charge and the other to take on a negative charge when the current is turned on. The negatively charged DNA fragments move toward the positive electrode.

As with the protein gel, the smaller the fragment, the more rapidly it moves through the gel. Small pieces of DNA will travel farther and be located farther from the well where they were initially injected. This allows the DNA fragments to form a distinct pattern that becomes visible through staining or a variety of other techniques.

The information provided by both DNA and protein gels looks very much like a bar code. The patterns formed from different protein samples or the DNA of different individuals can provide information about relatedness. DNA has been used to determine who the father and/or mother of a child actually is in paternity cases or in instances where a couple suspects that the child given to them in the hospital is not their child. It has also been used to determine guilt or innocence during criminal investigations. The source of blood, semen, or skin can be identified with this technique. DNA left at a crime scene can be compared to a suspect's DNA to determine if the suspect was at the crime scene.

In the case of endangered species, scientists can use DNA electrophoresis to learn which groups are being devastated by poachers, since skins from members of the same group will have similar DNA patterns. Gel electrophoresis can also be used to determine and to identify the genes responsible for specific genetic diseases such as sickle cell disease.

Chromatography Like gel electrophoresis, **chromatography** is a technique used for separating mixtures of molecules. In one type of chromatography commonly used in the biology laboratory, the mixture being separated is placed on a paper to which it sticks. For example, chlorophyll extract from plant leaves is placed on filter paper or special chromatography paper. It is done by placing a small dot of the concentrated chlorophyll extract near one end of a strip of the paper. Then, the end of the paper nearest the dot of extract is placed in a solvent. In the case of chlorophyll, the solvent could be alcohol. The solvent cannot touch the dot when it is initially set up, or the chlorophyll would simply wash away into the solvent.

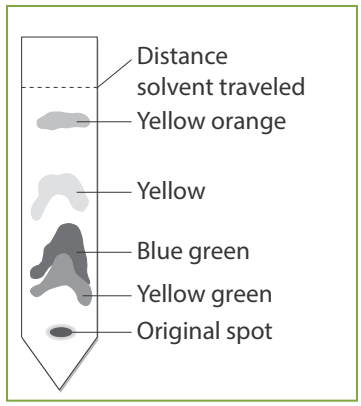


Figure 9-20. Chlorophyll chromatography

As the solvent soaks into the paper and moves upward, substances in the mixture that do not stick tightly to the paper will be picked up by the solvent and moved along quickly. Substances that are more tightly held to the paper and less attracted to the solvent will also be picked up but will move along more slowly. This results in the formation of bands of the different substances on the chromatography paper. If the substances in the mixture are colorless, they can be viewed by combining them with reactive chemicals that will give them color. The chlorophyll extract consists of several plant pigments that are very colorful and easy to distinguish.

In summary, the rate at which a substance moves along the paper in a given solvent can be used to separate it from other substances. By comparing the distances moved with those of known substances in the same solvent, the unknowns can be identified. See Figure 9-20.

Stains and Indicators **Stains** can be used to make cell structures more visible. In fact, chromosomes were so named for the fact that they are easily stained. Commonly used stains are iodine and methylene blue. Iodine darkens certain cell structures. It is especially useful when examining plant cells through the microscope. Methylene blue stains structures in the nucleus. It is useful when observing many types of cells.

An **indicator** is a substance that changes color when it contacts certain chemicals. The examples in Table 9-2 represent only a few of the indicators commonly used in the biology laboratory.

Other indicators can be used to determine how much sugar there is in a solution or the amount of carbon dioxide present. Swimming pool owners use indicators to tell them how much chlorine is in the water. Some of the pregnancy test kits that pharmacies sell also use indicators.

Table 9-2. Indicators Used in the Biology Laboratory

Indicator	What It Tests
pH paper	A piece of pH paper is dipped in the solution to be tested. Its color is then matched to a color scale. Specific colors indicate whether the solution is acidic (pH values from 0 to 6), neutral (pH 7), or basic (pH values from 8 to 14).
Iodine (Lugol's) solution	A color change from golden brown to blue-black indicates the presence of starch in the tested solution.

Dichotomous Keys A key is used to sort, name, and/or classify a particular organism. By working through a series of steps, organisms are eliminated until the one of interest is finally identified. Each step of a **dichotomous key** typically consists of two statements that divide the things being identified/classified into two groups. Each statement is followed by a direction that indicates either what step to go to next or the name of the organism.

To make your own dichotomous key, you would need to start out with two statements that divide the organisms being classified into two groups.

1a	Requires petroleum fuel	go to Step 2
1b	Requires only muscle power	bicycle
2a	Has wings and flies	jet
2b	Has no wings and does not fly	go to Step 3
3a	Has two wheels	motorcycle
3b	Has more than two wheels	car

Figure 9-21. A dichotomous key is used to sort, name, or classify.

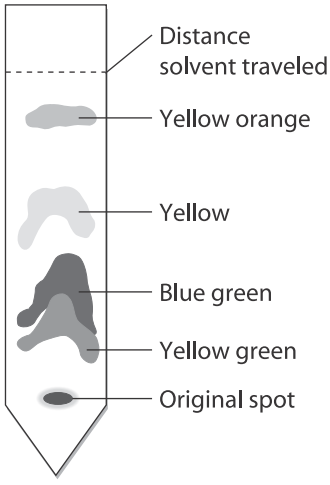
Each statement would be followed with a direction about the next step to take. At the next step, you again divide the organisms into two groups that are again followed by directions about the next step to go to or the identity of the organism or object. For example, Figure 9-21 shows how you might construct a key to classify a bicycle, car, jet, and motorcycle.

Pretend that you do not know a motorcycle from a bicycle from a jet, from a car. All you could do is examine each one of the objects, make observations, and determine how they work. Once you did that, you would be ready to place names on each of the four items.

Notice that if you did not work through the key step by step, it would be very easy to misname an object. If you thought about a bicycle as having two wheels and scanned down the list with no attention to following the steps, you could easily mistake the bicycle for a motorcycle. You would spot step 3a, which says, "Has two wheels" and say to yourself, "Yep, that's the bicycle." You would totally miss the fact that the object in step 3a must run on petroleum fuel. Always start at the beginning of a key.

Review Questions — Set 9.2

Base your answers to questions 9 through 11 on the diagram and information below, and on your knowledge of biology.



Several drops of concentrated green pigment extract obtained from spinach leaves were placed near the bottom of a strip of highly absorbent paper. When the extract dried, the paper was suspended in a test tube containing solvent so that only the tip of the paper was in the solvent. As the solvent was absorbed and moved up the paper, the various pigments within the extract became visible, as shown in the diagram.

9. A valid conclusion that can be drawn from this information is that spinach leaves

(1) contain only chlorophyll
(2) contain pigments in addition to chlorophyll
(3) contain more orange pigment than yellow pigment
(4) are yellow-orange rather than green

10. The technique used to separate the parts of the extract in the diagram is known as

(1) staining
(2) dissection
(3) chromatography
(4) electrophoresis

11. In which organelle would most of these pigments be found?

(1) nucleus
(2) mitochondrion
(3) ribosome
(4) chloroplast

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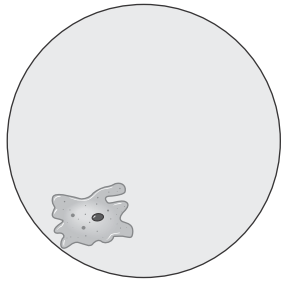
12. A student observed a one-celled organism in the field of view of a compound light microscope as shown in the adjacent diagram.

On the diagram, draw an arrow to indicate the direction the organism would seem to move if the student moved the slide on the stage to the left and down. [1]



13. To test for the presence of glucose, a student added the same amount of Benedict's solution to each of four test tubes. (Benedict's is a glucose indicator that is a royal blue color when no glucose is present. To determine if glucose is present, Benedict's must be mixed in the unknown solution and heated for several minutes.)

Two of the test tubes contained unknown solutions. The other two test tubes contained known solutions. The chart below shows the color results obtained after the solutions were heated in the four test tubes in a hot water bath.

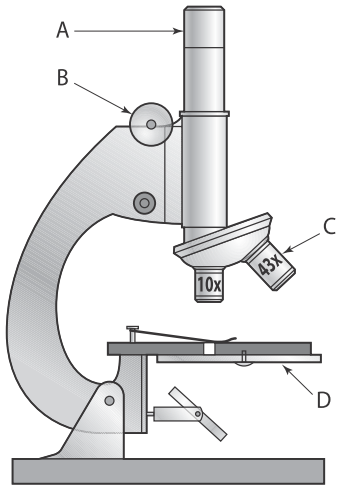
Data Table

Tube	Contents	Color After Heating
1	Unknown solution + Benedict's solution	Royal blue
2	Unknown solution + Benedict's solution	Red orange
3	Water + Benedict's solution	Royal blue
4	Glucose + water + Benedict's solution	Red orange

The student could correctly conclude that

(1) all of the tubes contained glucose
(2) tubes 1 and 2 contained glucose
(3) tube 1 did not contain glucose, but tube 2 did
(4) tube 2 did not contain glucose, but tube 1 did

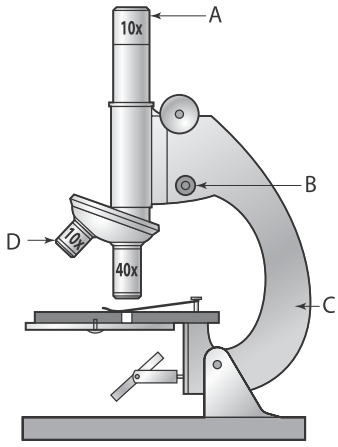
14. A student viewing a specimen under the low-power objective of a compound light microscope switched to high power and noticed that the field of view darkened considerably.



Which microscope part identified on this microscope would the student adjust to brighten the field of view?

(1) A (2) B (3) C (4) D

15. A student studied the upper layer of cells of a tissue sample on a slide, using the high-power objective of the compound microscope shown.



Which part of the microscope should the student adjust to observe the lower layer of the sample?

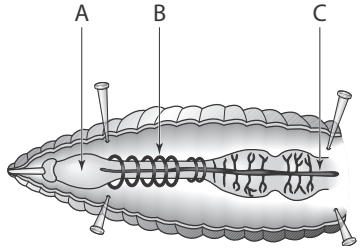
(1) A (2) B (3) C (4) D

16. State the purpose of using stains in a wet-mount slide preparation. [1]

17. The eyepiece of a compound light microscope has a magnification of 10X and the low-power objective and high-power objective lenses have magnifications of 10X and 30X, respectively. If the diameter of the low-power field measures 1500 micrometers, the diameter of the high-power field will measure either 4500 micrometers or 500 micrometers.

Select the correct diameter. Support your answer. [1]

Base your answers to questions 18 and 19 on the diagram below of some internal structures of an earthworm and on your knowledge of biology.



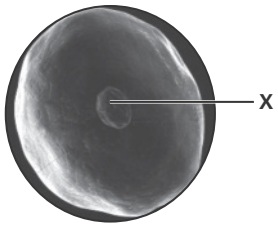
18. Which laboratory equipment should be used to observe the surface details of structures A, B, and C of the earthworm?

(1) stereoscope
(2) compound light microscope
(3) graduated cylinder
(4) triple-beam balance

19. Structure A has a diameter of 3 millimeters. What is the approximate diameter of the blood vessel indicated by C?

(1) 2.5 mm (3) 1.5 mm
(2) 2.0 mm (4) 0.5 mm

Base your answers to questions 20 and 21 on the illustration below and on your knowledge of biology. The image is a representation of an animal cell as it would appear when viewed with compound light microscope



20. Identify the organelle indicated by letter X. [1]

21. What technique could be used to make the organelle indicated by letter X more visible? [1]

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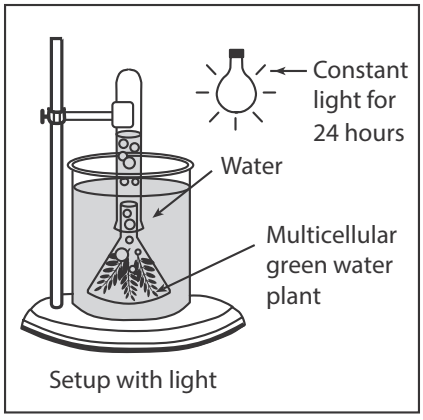
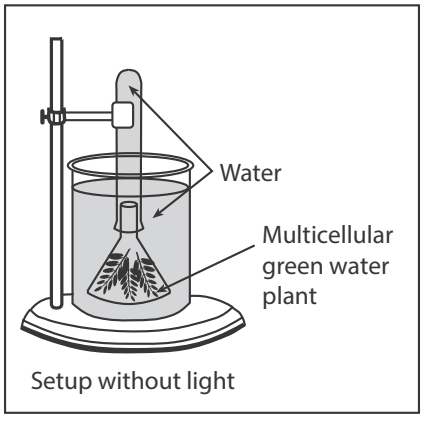
22. While focusing a microscope on high power, a student crushed the coverslip. The student probably

(1) shut the light off
(2) turned up the light intensity
(3) rotated the eyepiece
(4) used the coarse adjustment

23. An experimental setup is shown in the diagram below.

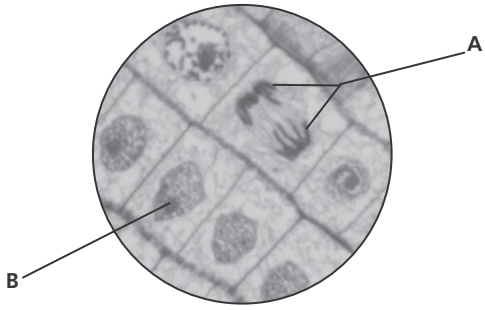




Which hypothesis would most likely be tested using this setup?

(1) Green water plants release a gas in the presence of light.
(2) Roots of water plants absorb minerals in the absence of light.
(3) Green plants need light for cell division.
(4) Plants grow best in the absence of light.

Base your answers to questions 24 through 27 on the photograph below and on your knowledge of biology. The photograph shows onion root-tip tissue viewed under the high-power objective of a compound light microscope.



24. The photograph illustrates stages in the process of

(1) meiosis in root tips
(2) mitotic cell division in plants
(3) water conduction in onions
(4) chlorophyll production in chloroplasts

25. Identify the structure indicated by arrow A. [1]

26. Identify the structure indicated by arrow B. [1]

27. Describe one adjustment that could be made to the microscope to make the field of view brighter. [1]

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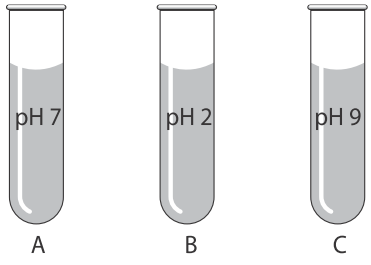
28. Pieces of pH paper were used to test the contents of three test tubes. The results are shown in the diagram below.



Which statement about the tubes is correct?

(1) Tube A contains a base.
(2) Tube B contains a base.
(3) Tube B contains an acid.
(4) Tube C contains an acid.

29. When viewed with a compound light microscope, which letter would best illustrate the way in which the microscope inverts and reverses the image?

(1) A (2) W (3) F (4) D

30. Gel electrophoresis is a technique used to

(1) cut DNA into pieces of various sizes
(2) separate DNA fragments by charge and size
(3) move DNA fragments from one species to another
(4) make copies of chromosomes

Observing Plant and Animal Specimens

Classroom experiences involving plants and animals range from observation to dissection. Opportunities to observe plants and animals require consideration and appreciation for the organism. The abuse of any live organism for any purpose is intolerable.

Dissection and Preserved Specimens

The dissection of plant and animal specimens provides a framework upon which to organize biological knowledge. **Dissection** (or the examination of preserved specimens) provides a way to

- observe similarities and differences that exist among species
- understand the relationship between biological form and function
- expose and identify the internal structures of organisms

To dissect a specimen correctly, you need:

- the knowledge of what equipment to use and how to use it properly
- a work area that is clean and well organized both before and after the activity

Equipment commonly used during dissection activities is described in Table 9-3.

Table 9-3. Dissection Equipment

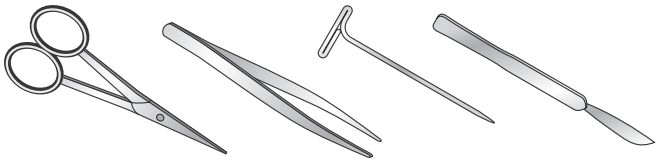
Equipment	Use
Dissecting pan	Resembles a cake pan but has a wax or a rubber-like substance in the bottom. The specimen is placed on the waxy surface.
Dissecting pins	Large pins with a "T" shape used to anchor the specimen during the dissection
Scalpel	A sharp instrument used to slice open the specimen so that the internal parts can be observed
Scissors	Used for cutting open the specimen and to remove parts. May have two sharp points or one blunt and one sharp point.
Probe/ Teasing needle/ Dissecting needle	Used to move structures around while they are still intact. The probe can be used to lift some organs so that others located below them are observable. The probe or dissecting needle is also used to point out different structures when showing specific features to someone else. Another function is to "tease" or gently tear apart structures such as muscle tissue.
Tweezers/ Forceps	Used to lift out small parts, to move structures, and to pry parts open
Safety goggles	Wrap-around shatter-proof glasses used to protect eyes from accidental splashes of preservative when dissecting as well as in other lab situations.

Review Questions — Set 9.3

31. State one scientific purpose for dissecting an organism. [1]

32. For what purpose would the equipment in the following illustration most likely be used?

(1) dissecting an earthworm
(2) removing cell organelles
(3) identifying and classifying a single-celled organism
(4) observing mitosis on prepared slides



33. Along with a dissecting pan, which group of equipment would be most useful to a student planning to dissect a preserved specimen?

(1) dissecting pins, compound light microscope, and pH paper
(2) pH paper, eye dropper, and safety goggles
(3) safety goggles, scissors, and compound light microscope
(4) safety goggles, stereoscope, and scissors

34. What is the best use for the dissection instrument illustrated in the diagram below?

(1) cutting through bones
(2) spreading apart muscle tissue
(3) cutting through thick muscles
(4) removing blood plasma

Laboratory Safety

Laboratory investigations are, for some students, the most exciting part of the Living Environment course. However, they sometimes involve potentially dangerous activities and materials. As a result, careful attention to safety procedures is critical.

Using Safety Equipment

You should know how to properly use the safety equipment located in your biology laboratory. It is critical to be aware of the location in your laboratory of each of these:

- fire extinguisher
- safety shower
- eye wash station
- fire blanket
- emergency gas shutoff

Use the safety equipment provided for you. Goggles should be worn whenever a lab calls for the use of chemicals, preserved specimens, or dissection. If indicated by your teacher, you should also wear a safety apron when using chemicals. Some activities, such as those involving chemicals or live or preserved organisms, also require the use of special gloves.

Safety in the Laboratory

Read all of the directions for an investigation before you start to work. If you are unsure about any part of the lab procedures, check with your teacher. As a general rule, do not perform activities without permission; do only what the instructions and your teacher direct you to do.

- Do *not* eat or drink in the laboratory.
- When you are heating a test tube, always slant it so that the open end of the tube points away from you and others. Never heat a closed container, such as a test tube that has been closed with a stopper.
- Never inhale or taste any of the chemicals you are using in a laboratory. This includes the specimens you are dissecting.
- If you spill a chemical or get any on your skin, wash it off immediately. Also, report the incident to your teacher.
- Tell your teacher about any personal injury no matter how minor it may seem.
- Tie back long hair, and keep loose clothing away from laboratory equipment, chemicals, and sources of heat and fire.
- Never expose flammable liquids to an open flame. Use a hot water bath (such as a large beaker of water heated on a hot plate) if you need to heat flammable liquids (such as alcohol). See Figure 9-22.
- Know what equipment to use when handling hot glassware. Do not use bare fingers to pick up hot test tubes or beakers. Use test tube holders and beaker tongs.
- Do not use glassware that has cracks or large chips. Tell your teacher about the damage and get a replacement.
- Do not pour chemicals back into stock bottles or exchange stoppers on the stock bottles.
- Use laboratory apparatus as it is intended to be used. For example, do not stir a solution using a thermometer, plastic ruler, or your pen.
- Do not use electrical equipment around water. If electrical cords seem to have exposed wires or if you get a shock handling electrical equipment, notify your teacher immediately. Do not attempt to disconnect the equipment yourself.

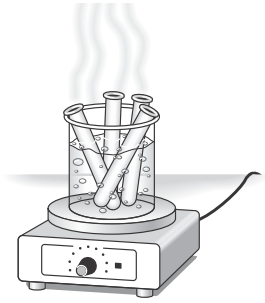


Figure 9-22. Hot water bath: A hot water bath is used to heat test tubes that contain a flammable liquid such as alcohol.

Cleaning Your Work Area

- Turn off the gas and water after you are done with them. Disconnect any electrical devices.
- Clean your work area by returning materials to their appropriate places, washing glassware according to your teacher's instructions, and wiping off the lab surface.
- Dispose of chemicals according to the instructions provided by your teacher.
- Wash your hands thoroughly!

Review Questions — Set 9.4

35. If a student spills nitric acid on her arm, she should *first*

(1) report the accident to the school nurse
(2) report the spill to her teacher
(3) rinse her arm with water
(4) allow the acid to evaporate

36. When they are not being used during a laboratory investigation, electrical devices should be

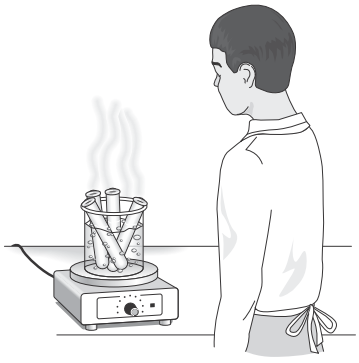
(1) put away
(2) turned off
(3) unplugged
(4) covered

37. A student performing an experiment noticed that the beaker containing the water being heated had a small crack. It was not leaking. What should the student do?

(1) Stop heating the beaker and try to fix the crack.
(2) Stop heating the beaker and report the crack to the teacher.
(3) Stop heating the beaker and immediately take the beaker to the teacher.
(4) Continue heating as long as the liquid does not start to leak out of the crack.

38. The diagram below shows a student conducting a laboratory experiment. Describe one safety procedure the student should be following that is *not* represented in the diagram. [1]

39. The diagram below shows a student heating some test tubes with chemicals in them during a laboratory activity. Describe one error in the laboratory procedure shown in the diagram. [1]



40. An *unsafe* procedure for heating a nutrient solution in a flask would be to

(1) heat the solution at the lowest temperature on a hot plate
(2) stopper the flask tightly to prevent evaporation of the solution
(3) use a Bunsen burner to heat the solution
(4) stir the solution while it is heating

41. Chlorophyll can be removed from leaves by boiling them in alcohol, a flammable solvent. In addition to wearing safety goggles, which is the safest procedure to follow?

(1) A stoppered test tube of leaves and alcohol should be held over the Bunsen burner.
(2) A stoppered test tube of leaves and alcohol should be placed in a large beaker of alcohol and heated on a hot plate.
(3) A beaker of leaves and alcohol should be placed on a tripod over a Bunsen burner.
(4) A beaker of leaves and alcohol should be placed into a larger beaker of water and heated on a hot plate.

42. Which safety procedure should a student follow during a dissection?

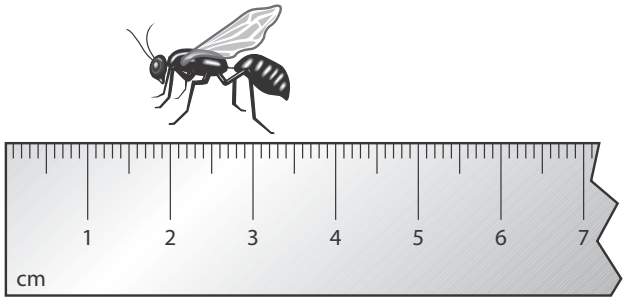
(1) The student should wear gloves and hold the specimen in the palm of her hand while cutting the specimen open.
(2) The student should cut the specimen open while holding it under running water.
(3) The student should apply additional preservative to the specimen.
(4) The student should direct the cutting motion away from her body.

Directions

Review the Test-Taking Strategies section of this book. Then answer the following questions. Read each question carefully and answer with a correct choice or response.

Part A

1 The diagram below shows a wasp positioned next to a centimeter ruler.



What is the approximate length of a *wing* of this wasp?

(1) 10 mm
(2) 1.4 cm
(3) 3.5 cm
(4) 35 mm

2 When preparing a wet-mount slide of onion cells, a student put a drop of Lugol's iodine stain on the slide. Lugol's iodine stain was added to

(1) prevent the formation of air bubbles
(2) make cell structures more visible
(3) increase the magnification
(4) increase the rate of photosynthesis in the cells

3 A student views some cheek cells under low power. Before switching to high power, the student should

(1) adjust the eyepiece
(2) center the image being viewed
(3) remove the slide from the stage
(4) remove the coverslip from the slide

4 Bromthymol blue turns yellow in the presence of carbon dioxide. This characteristic makes it possible for bromthymol blue to function as

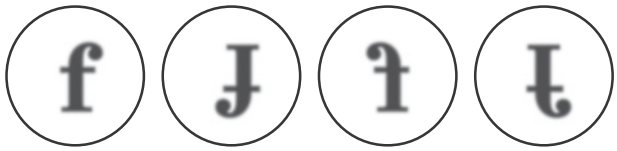
(1) a measure of volume
(2) an indicator
(3) a catalyst
(4) an energy source

5 Which statement describes two unsafe laboratory practices represented in the diagram below?

(1) The flame is too high, and the test tube is unstoppered.
(2) The opening of the test tube is pointed toward the student, and the student is not wearing goggles.
(3) The test tube is unstoppered, and the student is not wearing goggles.
(4) The beaker has water in it, and the flame is under the tripod.

6 A student sees the image shown below when observing the letter "f" with the low-power objective lens of a microscope.

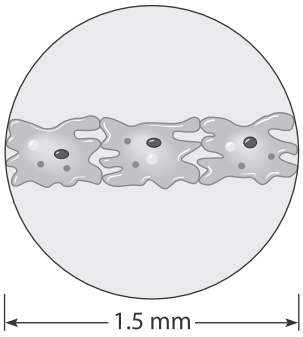
Which of the four diagrams below most closely resembles the image the student will see after switching to high power?



(1) (2) (3) (4)

Part B

7 The diagram below represents the field of view of a compound light microscope. Three single-celled organisms are located across the diameter of the field.



Knowing that 1 mm = 1000 micrometers, what is the approximate length of each single-celled organism?

(1) 250 micrometers
(2) 500 micrometers
(3) 1000 micrometers
(4) 1500 micrometers

8 Electrophoresis is a method of

(1) separating DNA fragments
(2) changing the genetic code of an organism
(3) indicating the presence of starch
(4) separating colored compounds on a strip of paper

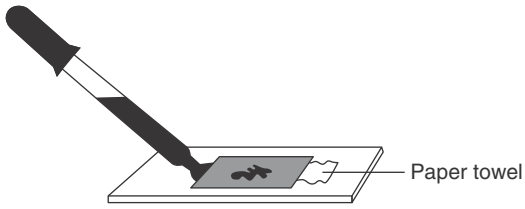
9 A student, not wearing safety goggles, gets some unknown chemical in his eye. What is the most appropriate action he should take?

(1) Put on safety goggles immediately.
(2) Ask his lab partner to see if his eye looks OK.
(3) Go to the eyewash station and use it to rinse his eye thoroughly.
(4) Rub the eye gently and see if it hurts or stings.

10 An indicator for a protein is added to a solution that contains protein and to a solution that does not contain protein. State one way, other than the presence or absence of protein, that the two solutions may differ after the indicator has been added to both. [1]

11 State two safety procedures that should be followed when conducting an experiment that involves heating protein in a test tube containing water, an acid, and a digestive enzyme. [1]

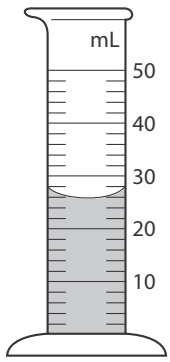
12 Which laboratory procedure is represented in the diagram below?



(1) placing a coverslip over a specimen
(2) removing a coverslip from a slide
(3) adding stain to a slide without removing the coverslip
(4) reducing the size of air bubbles under a coverslip

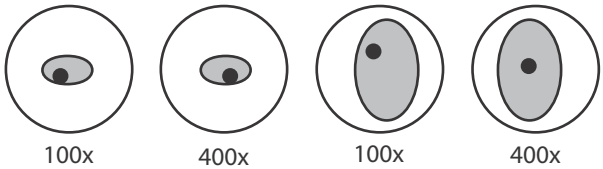
13 A student is viewing a single-celled organism under the low-power objective of a compound light microscope. Describe an adjustment the student would need to make to see the organism clearly *after* switching from low power to high power. In your description include the name of the part of the microscope that would be used to make the adjustment. [1]

14 What is the volume of the liquid in the graduated cylinder shown below?



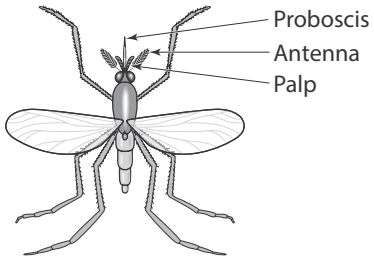
(1) 23 mL (2) 26 mL (3) 27 mL (4) 28 mL

15 The diagrams below show four different one-celled organisms (shaded) in the field of view of the same microscope using different magnifications. Which illustration shows the largest one-celled organism?

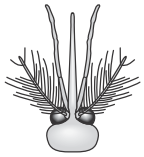
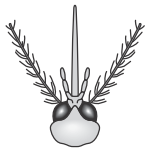
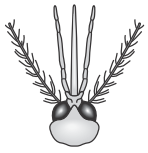
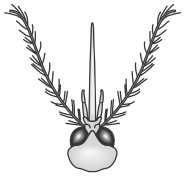
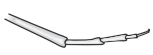


Base your answers to questions 16 through 18 on the information below, the key, and on your knowledge of biology.

Biologists use keys to accurately classify unknown organisms such as the unidentified female mosquito shown in the following diagram. These keys are designed to categorize organisms according to structural characteristics. The key shows various characteristics used to identify the differences among *Anopheles, Deinocerites, Culex, Psorophora,* and *Aedes mosquitoes.*



Unknown female mosquito

1a Antennae very bushy — Male mosquito 	1b Antennae not bushy — Go to 2 
2a Palps much shorter than proboscis — Go to 3 	2b Palps as long as proboscis — Female *Anopheles* 
3a Tip of abdomen blunt, without points — Go to 4 	3b Tip of abdomen with points — Go to 5 
4a Antennae much longer than proboscis — Female *Deinocerites* 	4b Antennae shorter than proboscis — Female *Culex* 
5a Many long scales present on hind legs — Female *Psorophora* 	5b Hind legs without long scales — Female *Aedes* 

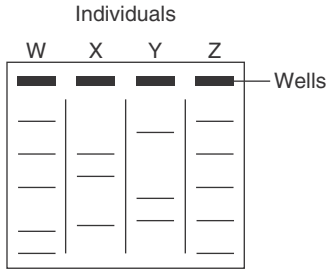
16 According to the key, which feature distinguishes male from female mosquitoes?
(1) palp length
(2) leg scales
(3) abdomen points
(4) antennae appearance

17 According to the key, which characteristics are necessary to identify a female *Anopheles* mosquito?
(1) antennae, palps, and proboscis
(2) wings, proboscis, and scales on legs
(3) eyes, scales on legs, and abdomen tip
(4) palps, abdomen tip, and wings

18 According to the key, the unknown female mosquito belongs to the group known as
(1) *Deinocerites*
(2) *Culex*
(3) *Psorophora*
(4) *Aedes*

Base your answers to questions 19 through 22 on the information and diagram below and on your knowledge of biology. The diagram represents some of the steps in a procedure used in a specific laboratory activity.

Samples of DNA from an eye-color gene of four individuals, W, X, Y, and Z, were cut into pieces using a type of chemical. The results of this procedure are shown below.



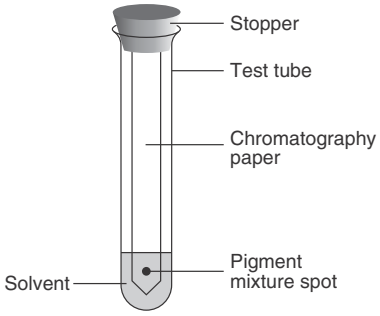
19 Identify the specific type of chemical used to cut the DNA in this procedure. [1]

20 Which two individuals have DNA base patterns for this gene that are the most similar? Support your answer. [1]

21 The diagram represents the results of the procedure known as
(1) cloning
(2) chromatography
(3) gel electrophoresis
(4) protein sequencing

22 State where the smallest fragments of DNA would be located on the gel in the illustration. [1]

23 A chromatography setup is shown below.



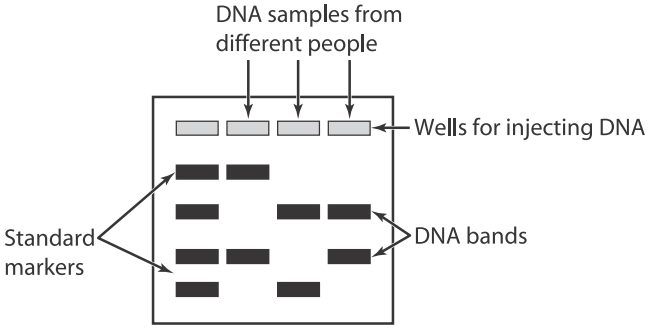
Identify one error in the setup. [1]

Part C

24 Some students did a lab to test the vitamin C content of several fruits. They squeezed the juice from some of the fruits and cut others up and placed them in a blender to obtain a juice sample. Juice for each fruit was kept in a clean, labeled beaker. Pipettes were used to transfer the juices to test tubes for analysis.

During the laboratory cleanup, one student drank some of the juice left in one beaker. State why this was an unsafe procedure. [1]

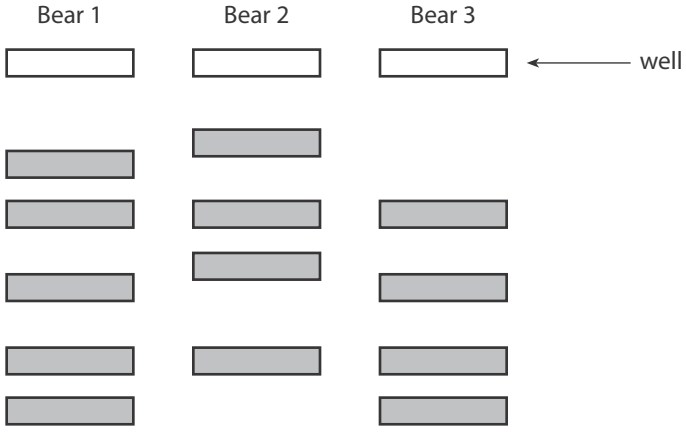
25 Below is a drawing of a hypothetical electrophoresis gel. Included on the gel are some bands for several different individuals.



- Decribe where the smallest fragments of DNA would be located on the gel in the illustration. [1]
- Discuss two practical applications of information that can be obtained through this process. [1]

Base your answers to questions 26 through 28 on the information and diagram below and on your knowledge of biology.

The diagram below shows the results of a test that was done using DNA samples from three bears of different species. Each DNA sample was cut into fragments using a specific enzyme and placed in the wells as indicated below. The DNA fragments were then separated using gel electrophoresis.



26 Which two bears are most closely related? Support your answer with data from the test results. [1]

27 Identify one additional way to determine the evolutionary relationship of these bears. [1]

28 Identify one procedure, other than electrophoresis, that is used in the laboratory to separate the different types of molecules in a liquid mixture. [1]

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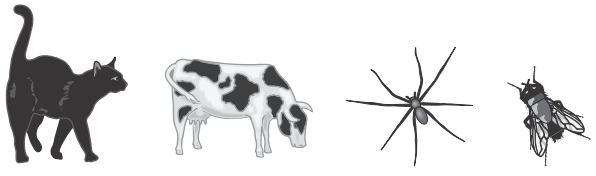
29 The dichotomous key begun below should allow users to classify the organisms illustrated. Complete the key using only information shown in the illustration. [3]



Dichotomous Key
1a. Wings present ..... Fly 1b. No wings present ..... go to 2