

Blood

Objectives

- ☐ Name the two major components of blood, and state their average percentages in whole blood.
- ☐ Describe the composition and functional importance of plasma.
- ☐ Define *formed elements*, and list the cell types composing them, state their relative percentages, and describe their major functions.
- ☐ Identify erythrocytes, basophils, eosinophils, monocytes, lymphocytes, and neutrophils when provided with a microscopic preparation or appropriate image.
- ☐ Provide the normal values for a total white blood cell count and a total red blood cell count, and state the importance of these tests.
- Conduct the following blood tests in the laboratory, and state their norms and the importance of each: differential white blood cell count, hematocrit, hemoglobin determination, clotting time, and plasma cholesterol concentration.
- Define leukocytosis, leukopenia, leukemia, polycythemia, and anemia; cite a possible cause for each condition.
- Perform an ABO and Rh blood typing test in the laboratory, and discuss the reason for transfusion reactions resulting from the administration of mismatched blood.

Materials

General supply area:

- Disposable gloves
- Safety glasses (student-provided)
- Bucket or large beaker containing 10% household bleach solution for slide and glassware disposal
- Spray bottles containing 10% bleach solution
- Autoclave bag
- Designated lancet (sharps) disposal container

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Pre-Lab Quiz

- 1. Circle True or False. There are no special precautions that I need to observe when performing today's lab.
- 2. Three types of formed elements found in blood include erythrocytes, leukocytes, and:
 - a. electrolytes
- b. fibers
- c. platelets
- d. sodium salts
- 3. Circle the correct underlined term. Mature erythrocytes / leukocytes are the most numerous blood cells and do not have a nucleus.
- 4. The least numerous but largest of all agranulocytes is the:
 - a. basophil
- **b.** lymphocyte **c.** monocyte
- d. neutrophil
- are the leukocytes responsible for releasing histamine and other mediators of inflammation.
 - a. Basophils
- b. Eosinophils c. Monocytes
- d. Neutrophils
- are essential for blood clotting.
- 7. Circle the correct underlined term. When determining the hematocrit / hemoglobin, you will centrifuge whole blood in order to allow the formed elements to sink to the bottom of the sample.
- 8. Circle the correct underlined term. The normal hematocrit value for females / males is generally higher than that of the opposite sex.
- 9. Circle the correct underlined term. Blood typing is based on the presence of proteins known as antigens / antibodies on the outer surface of the red blood cell plasma membrane.
- 10. Circle True or False. If an individual is transfused with the wrong blood type, the recipient's antibodies react with the donor's blood antigens, eventually clumping and hemolyzing the donated RBCs.

- Plasma (obtained from an animal hospital or prepared by centrifuging animal [for example, cattle or sheep] blood obtained from a biological supply house)
- Test tubes and test tube racks
- Wide-range pH paper
- Stained smears of human blood from a biological supply house or, if desired by the instructor, heparinized animal blood obtained from a biological supply house or an animal hospital (for example, dog blood), or EDTA-treated red cells (reference cells*) with blood type labels obscured (available from Immucor, Inc.)

Note to the Instructor: See directions below for handling of soiled glassware and disposable items.

*The blood in these kits (each containing four blood cell types—A1, A2, B, and O individually supplied in 10-ml vials) is used to calibrate cell counters and other automated clinical laboratory equipment. This blood has been carefully screened and can be safely used by students for blood typing and determining hematocrits. It is not usable for hemoglobin determinations or coagulation studies.

- · Clean microscope slides
- · Glass stirring rods
- Wright's stain in a dropper bottle

- Distilled water in a dropper bottle
- Sterile lancets
- Absorbent cotton balls
- Alcohol swabs (wipes)
- Paper towels
- Compound microscope
- Immersion oil
- Assorted slides of white blood count pathologies labeled "Unknown Sample
- Timer

Because many blood tests are to be conducted in this exercise, it is advisable to set up a number of appropriately labeled supply areas for the various tests, as designated below. Some needed supplies are located in the general supply area.

Note: Artificial blood prepared by Ward's Natural Science can be used for differential counts, hematocrit, and blood typing.

Activity 4: Hematocrit

- Heparinized capillary tubes
- Microhematocrit centrifuge and reading gauge (if the reading gauge is not available, a millimeter ruler may be
- Capillary tube sealer or modeling clay

Activity 5: Hemoglobin determination

 Hemoglobinometer, hemolysis applicator. and lens paper; or Tallquist hemoglobin scale and test paper

Activity 6: Coagulation time

- Capillary tubes (nonheparinized)
- Fine triangular file

Activity 7: Blood typing

- · Blood typing sera (anti-A, anti-B, and anti-Rh [anti-D])
- Rh typing box
- Wax marking pencil
- Toothpicks
- Medicine dropper
- Blood test cards or microscope slides

Activity 8: Demonstration

 Microscopes set up with prepared slides demonstrating the following bone (or bone marrow) conditions: macrocytic hypochromic anemia, microcytic hypochromic anemia, sickle cell anemia, lymphocytic leukemia (chronic), and eosinophilia

Activity 9: Cholesterol measurement

Cholesterol test cards and color scale



PEx PhysioEx™ 9.1 Computer Simulation Ex. 11 on p. PEx-161.

n this exercise, you will study plasma and formed elements of blood and conduct various hematologic tests. These tests are useful diagnostic tools for the physician because blood composition (number and types of blood cells, and chemical composition) reflects the status of many body functions and malfunctions.

ALERT: Special precautions when handling blood. This exercise provides information on blood from several sources: human, animal, human treated, and artificial blood. The instructor will decide whether to use animal blood for testing or to have students test their own blood in accordance with the educational goals of the student group. For example, for students in the nursing or laboratory technician curricula, learning how to safely handle human blood or other human wastes is essential. Whenever blood is being handled, special attention must be paid to safety precautions. Instructors who opt to use human blood are responsible for its safe handling. Precautions should be used regardless of the source of the blood. This will both teach good technique and ensure the safety of the students. Follow exactly the safety precautions listed below.

- 1. Wear safety gloves at all times. Discard appropriately.
- 2. Wear safety glasses throughout the exercise.
- 3. Handle only your own, freshly drawn (human) blood.
- 4. Be sure you understand the instructions and have all supplies on hand before you begin any part of the exercise.
- 5. Do not reuse supplies and equipment once they have been exposed to blood.
- 6. Keep the lab area clean. Do not let anything that has come in contact with blood touch surfaces or other individuals in the lab. Keep track of the location of any supplies and equipment that come into contact with blood.
- 7. Immediately after use dispose of lancets in a designated disposal container. Do not put them down on the lab bench, even temporarily.
- 8. Dispose of all used cotton balls, alcohol swabs, blotting paper, and so forth, in autoclave bags; place all soiled glassware in containers of 10% bleach solution.
- 9. Wipe down the lab bench with 10% bleach solution when you finish.

Composition of Blood

Circulating blood is a rather viscous substance that varies from bright red to a dull brick red, depending on the amount of oxygen it is carrying. Oxygen-rich blood is bright red. The average volume of blood in the body is about 5–6 L in adult males and 4–5 L in adult females.

Blood is classified as a type of connective tissue because it consists of a nonliving fluid matrix (the **plasma**) in which living cells (**formed elements**) are suspended. The fibers typical of a connective tissue matrix become visible in blood only when clotting occurs. They then appear as fibrin threads, which form the structural basis for clot formation.

More than 100 different substances are dissolved or suspended in plasma (**Figure 29.1**), which is over 90% water. These include nutrients, gases, hormones, various wastes and

metabolites, many types of proteins, and electrolytes. The composition of plasma varies continuously as cells remove or add substances to the blood.

Three types of formed elements are present in blood (Table 29.1, p. 428). Most numerous are erythrocytes, or red blood cells (RBCs), which are literally sacs of hemoglobin molecules that transport the bulk of the oxygen carried in the blood (and a small percentage of the carbon dioxide). Leukocytes, or white blood cells (WBCs), are part of the body's nonspecific defenses and the immune system, and platelets function in hemostasis (blood clot formation); together they make up <1% of whole blood. Formed elements normally constitute about 45% of whole blood; plasma accounts for the remaining 55%.

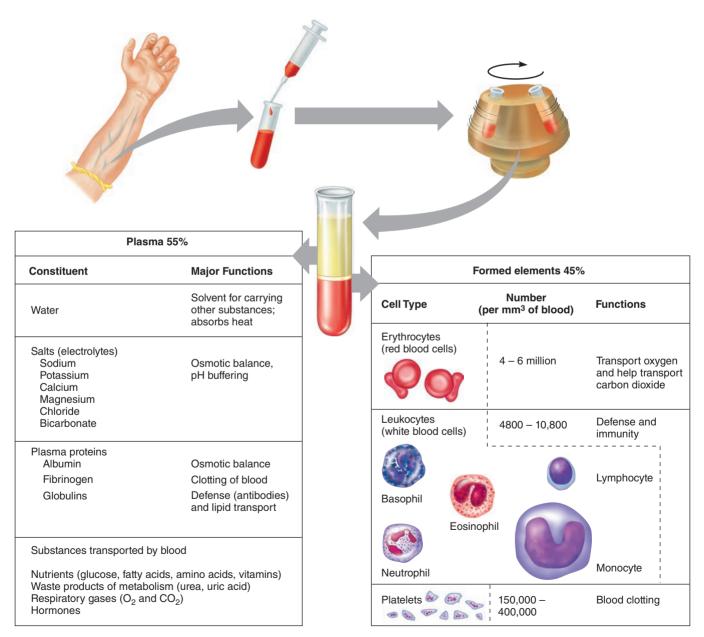


Figure 29.1 The composition of blood. Note that leukocytes and platelets are found in the band between plasma (above) and erythrocytes (below).

Cell type	Illustration	Description*	Cells/mm³ (µI) of blood	Function
Erythrocytes (red blood cells, RBCs)		Biconcave, anucleate disc; orange-pink color; diameter 7–8 µm	4–6 million	Transport oxygen and carbon dioxide
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800	
Granulocytes Neutrophil		Nucleus multilobed; pale red and blue cytoplasmic granules; diameter 10–12 μm	3000–7000 Differential count: 50–70%	Phagocytize pathogens or debris
Eosinophil	0	Nucleus bilobed; red cytoplasmic granules; diameter 10–14 µm	100–400 Differential count: 2–4%	Kill parasitic worms; slightly phagocytic; complex role in allergy and asthma
Basophil		Nucleus lobed; large blue- purple cytoplasmic granules; diameter 10–14 µm	20–50 Differential count: <1%	Release histamine and other mediators of inflammation; contain heparin, an anticoagulant
Agranulocytes Lymphocyte		Nucleus spherical or indented; pale blue cytoplasm; diameter 5–17 µm	1500–3000 Differential count: 20–40%	Mount immune response by direct cell attack or via antibody production
Monocyte		Nucleus U- or kidney- shaped; gray-blue cytoplasm; diameter 14–24 µm	100–700 Differential count: 3–8%	Develop into macrophages in tissues and phagocytize pathogens or debris
Platelets		Cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 µm	150,000–400,000	Seal small tears in blood vessels; instrumental in blood clotting

^{*}Appearance when stained with Wright's stain.

Activity 1

Determining the Physical Characteristics of Plasma

Go to the general supply area and carefully pour a few milliliters of plasma into a test tube. Also obtain some wide-range pH paper, and then return to your laboratory bench to make the following simple observations.

pH of Plasma

Test the pH of the plasma with wide-range pH paper.

Record the pH observed.

Color and Clarity of Plasma

Hold the test tube up to a source of natural light. Note and record its color and degree of transparency. Is it clear, translucent, or opaque?

Degree	of	trans	parenc	V
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Consistency

While wearing gloves, dip your finger and thumb into plasma, and then press them firmly together for a few seconds. Gently pull them apart. How would you describe the consistency of plasma (slippery, watery, sticky, granular)? Record your observations.

Activity 2

Examining the Formed Elements of Blood Microscopically

In this section, you will observe blood cells on an already prepared (purchased) blood slide or on a slide prepared from your own blood or blood provided by your instructor.

- If you are using the purchased blood slide, obtain a slide and begin your observations at step 6.
- If you are testing blood provided by a biological supply source or an animal hospital, obtain a tube of the supplied blood, disposable gloves, and the supplies listed in step 1, except for the lancets and alcohol swabs. After donning gloves, go to step 3b to begin your observations.
- If you are examining your own blood, you will perform all the steps described below *except* step 3b.
- 1. Obtain two glass slides, a glass stirring rod, dropper bottles of Wright's stain and distilled water, two or three lancets, cotton balls, and alcohol swabs. Bring this equipment to the laboratory bench. Clean the slides thoroughly and dry them.
- 2. Open the alcohol swab packet, and scrub your third or fourth finger with the swab. (Because the pricked finger may be a little sore later, it is better to prepare a finger on the nondominant hand.) Swing your hand in a coneshaped path for 10 to 15 seconds. This will dry the alcohol and cause your fingers to become filled with blood. Then, open the lancet packet and grasp the lancet by its blunt end. Quickly jab the pointed end into the prepared finger to produce a free flow of blood. It is *not* a good idea to squeeze or "milk" the finger, because this forces out tissue fluid as well as blood. If the blood is not flowing freely, make another puncture.

<u>Under no circumstances is a lancet to be used for more than one puncture.</u> Dispose of the lancets in the designated disposal container immediately after use.

3a. With a cotton ball, wipe away the first drop of blood; then allow another large drop of blood to form. Touch the blood to one of the cleaned slides approximately 1.3 cm, or ½ inch, from the end. Then quickly (to prevent clotting) use the second slide to form a blood smear (**Figure 29.2**). When properly prepared, the blood smear is uniformly thin. If the blood smear appears streaked, the blood probably began to clot or coagulate before the smear was made, and another slide should be prepared. Continue at step 4.

- 3b. Dip a glass rod in the blood provided, and transfer a generous drop of blood to the end of a cleaned microscope slide. For the time being, lay the glass rod on a paper towel on the bench. Then, as described in step 3a (Figure 29.2), use the second slide to make your blood smear.
- 4. Allow the blood smear slide to air dry. When it is completely dry, it will look dull. Place it on a paper towel, and add 5 to 10 drops of Wright's stain. Count the number of drops of stain used. Allow the stain to remain on the slide for 3 to 4 minutes, and then add an equal number of drops of distilled water. Allow the water and Wright's stain mixture to remain on the slide for 4 or 5 minutes or until a metallic green film or scum is apparent on the fluid surface.
- 5. Rinse the slide with a stream of distilled water. Then flood it with distilled water, and allow it to lie flat until the slide becomes translucent and takes on a pink cast. Then stand the slide on its long edge on the paper towel, and

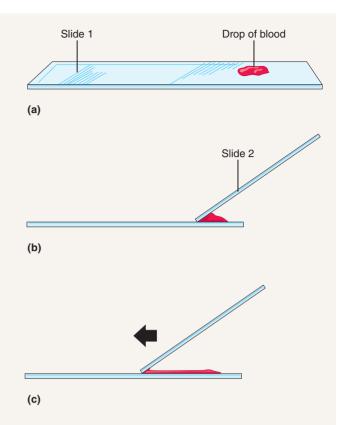


Figure 29.2 Procedure for making a blood smear.

(a) Place a drop of blood on slide 1 approximately ½ inch from one end. (b) Hold slide 2 at a 30° to 40° angle to slide 1 (it should touch the drop of blood) and allow blood to spread along entire bottom edge of angled slide. (c) Smoothly advance slide 2 to end of slide 1 (blood should run out before reaching the end of slide 1). Then lift slide 2 away from slide 1 and place slide 1 on a paper towel. Dispose of slide 2 in the appropriate container.

allow it to dry completely. Once the slide is dry, you can begin your observations.

- 6. Obtain a microscope and scan the slide under low power to find the area where the blood smear is the thinnest. After scanning the slide in low power to find the areas with the largest numbers of WBCs, read the following descriptions of cell types, and find each one in the art illustrating blood cell types (in Figure 29.1 and Table 29.1). (The formed elements are also shown in **Figure 29.3**, p. 430, and Figure 29.4.) Then, switch to the oil immersion lens, and observe the slide carefully to identify each cell type.
- **7.** Set your prepared slide aside for use in Activity 3.

Erythrocytes

Erythrocytes, or red blood cells, which average 7.5 μm in diameter, vary in color from an orange-pink color to pale pink, depending on the effectiveness of the stain. They have a distinctive biconcave disc shape and appear paler in the center than at the edge (see Figure 29.3).

As you observe the slide, notice that the red blood cells are by far the most numerous blood cells seen in the

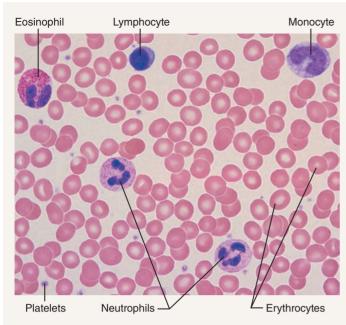


Figure 29.3 Photomicrograph of a human blood smear stained with Wright's stain (765×).

field. Their number averages 4.5 million to 5.5 million cells per cubic millimeter of blood (for women and men, respectively).

Red blood cells differ from the other blood cells because they are anucleate (lacking a nucleus) when mature and circulating in the blood. As a result, they are unable to reproduce or repair damage and have a limited life span of 100 to 120 days, after which they begin to fragment and are destroyed, mainly in the spleen.

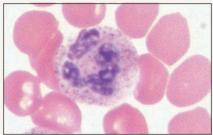
In various anemias, the red blood cells may appear pale (an indication of decreased hemoglobin content) or may be nucleated (an indication that the bone marrow is turning out cells prematurely).

Leukocytes

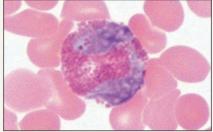
Leukocytes, or white blood cells, are nucleated cells that are formed in the bone marrow from the same stem cells (hemocytoblast) as red blood cells. They are much less numerous than the red blood cells, averaging from 4800 to 10,800 cells per cubic millimeter. The life span of leukocytes varies. They can survive for minutes or decades, depending on the type of leukocyte and tissue activity. Basically, white blood cells are protective, pathogendestroying cells that are transported to all parts of the body in the blood or lymph. Important to their protective function is their ability to move in and out of blood vessels, a process called diapedesis, and to wander through body tissues by amoeboid motion to reach sites of inflammation or tissue destruction. They are classified into two major groups, depending on whether or not they contain conspicuous granules in their cytoplasm.

Granulocytes make up the first group. The granules in their cytoplasm stain differentially with Wright's stain, and they have peculiarly lobed nuclei, which often consist of expanded nuclear regions connected by thin strands of nucleoplasm. There are three types of granulocytes: **neutrophils**, **eosinophils**, and **basophils**.

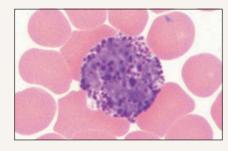
The second group, **agranulocytes**, contains no *visible* cytoplasmic granules. Although found in the bloodstream, they are much more abundant in lymphoid tissues. There are two types of agranulocytes: **lymphocytes** and **monocytes**. The specific characteristics of leukocytes are described in Table 29.1. Photomicrographs of the leukocytes illustrate their different appearances (**Figure 29.4**).



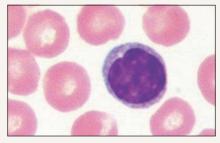
(a) Neutrophil; multilobed nucleus, pale red and blue cytoplasmic granules



(b) Eosinophil; bilobed nucleus, red cytoplasmic granules



(c) Basophil; bilobed nucleus, purplish black cytoplasmic granules



(d) Small lymphocyte; large spherical nucleus



(e) Monocyte; kidney-shaped nucleus

Figure 29.4 Leukocytes. In each case, the leukocytes are surrounded by erythrocytes (1330×, Wright's stain).

Students are often asked to list the leukocytes in order from the most abundant to the least abundant. The following silly phrase may help you with this task: *Never let monkeys eat bananas* (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

Platelets

Platelets are cell fragments of large multinucleate cells (megakaryocytes) formed in the bone marrow. They appear as darkly staining, irregularly shaped bodies interspersed

among the blood cells (see Figure 29.3). The normal platelet count in blood ranges from 150,000 to 400,000 per cubic millimeter. Platelets are instrumental in the clotting process that occurs in plasma when blood vessels are ruptured.

After you have identified these cell types on your slide, observe charts and three-dimensional models of blood cells if these are available. Do not dispose of your slide, as you will use it later for the differential white blood cell count.

Hematologic Tests

When someone enters a hospital as a patient, several hematologic tests are routinely done to determine general level of health as well as the presence of pathologic conditions. You will be conducting the most common of these tests in this exercise.

Materials such as cotton balls, lancets, and alcohol swabs are used in nearly all of the following diagnostic tests. These supplies are at the general supply area and should be properly disposed of (glassware to the bleach bucket, lancets in a designated disposal container, and disposable items to the autoclave bag) immediately after use.

Other necessary supplies and equipment are at specific supply areas marked according to the test with which they are used. Since nearly all of the tests require a finger stick, if you will be using your own blood it might be wise to quickly read through the tests to determine in which instances more than one preparation can be done from the same finger stick. A little planning will save you the discomfort of multiple finger sticks.

An alternative to using blood obtained from the finger stick technique is using heparinized blood samples supplied by your instructor. The purpose of using heparinized tubes is to prevent the blood from clotting. Thus blood collected and stored in such tubes will be suitable for all tests except coagulation time testing.

Total White and Red Blood Cell Counts

A **total WBC count** or **total RBC count** determines the total number of that cell type per unit volume of blood. Total WBC and RBC counts are a routine part of any physical exam. Most clinical agencies use computers to conduct these counts. Total WBC and RBC counts will not be done here, but the importance of such counts (both normal and abnormal values) is briefly described below.

Total White Blood Cell Count

Since white blood cells are an important part of the body's defense system, it is essential to note any abnormalities in them.

Leukocytosis, an abnormally high WBC count, may indicate bacterial or viral infection, metabolic disease, hemorrhage, or poisoning by drugs or chemicals. A decrease in the white cell number below 4000/mm³ (leukopenia) may indicate infectious hepatitis or cirrhosis, tuberculosis, or excessive antibiotic or X-ray therapy. A person with leukopenia lacks the usual protective mechanisms. Leukemia, a

malignant disorder of the lymphoid tissues characterized by uncontrolled proliferation of abnormal WBCs accompanied by a reduction in the number of RBCs and platelets, is detectable not only by a total WBC count but also by a differential WBC count.

Total Red Blood Cell Count

Since RBCs are absolutely necessary for oxygen transport, a doctor typically investigates any excessive change in their number immediately.

An increase in the number of RBCs (polycythemia) may result from bone marrow cancer or from living at high altitudes where less oxygen is available. A decrease in the number of RBCs results in anemia. The term anemia simply indicates a decreased oxygen-carrying capacity of blood that may result from a decrease in RBC number or size or a decreased hemoglobin content of the RBCs. A decrease in RBCs may result suddenly from hemorrhage or more gradually from conditions that destroy RBCs or hinder RBC production. +

Differential White Blood Cell Count

To make a **differential white blood cell count,** 100 WBCs are counted and classified according to type. Such a count is routine in a physical examination and in diagnosing illness, since any abnormality in percentages of WBC types may indicate a problem and the source of pathology.

Activity 3

Conducting a Differential WBC Count

- 1. Use the slide prepared for the identification of the blood cells in Activity 2 or a prepared slide provided by your instructor. Begin at the edge of the smear and move the slide in a systematic manner on the microscope stage—either up and down or from side to side (as indicated in **Figure 29.5** on p. 432).
- 2. Record each type of white blood cell you observe by making a count in the first blank column of the **Activity**3 chart on p. 432 (for example, ||| = 7 cells) until you have observed and recorded a total of 100 WBCs. Using the following equation, compute the percentage of each WBC type counted, and record the percentages on

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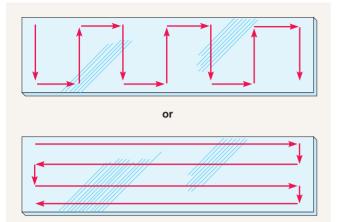


Figure 29.5 Alternative methods of moving the slide for a differential WBC count.

the Hematologic Test Data Sheet on the last page of the exercise, preceding the Review Sheet.

Percent (%) =
$$\frac{\text{# observed}}{\text{Total # counted}} \times 100$$

3. Select a slide marked "Unknown sample," record the slide number, and use the count chart below to conduct a differential count. Record the percentages on the data sheet (p. 438).

How does the differential count from the unknown sample slide compare to the normal percentages given for each type in Table 29.1?

Activity 3: Count of 100 WBCs			
Number observed			
Cell type	Student blood smear	Unknown sample #	
Neutrophils			
Eosinophils			
Basophils			
Lymphocytes			
Monocytes			

Using the text and other references, try to determine the blood pathology on the unknown slide. Defend your answer.

4. How does your differential white blood cell count compare to the percentages given in Table 29.1?

Hematocrit

The **hematocrit** is routinely determined when anemia is suspected. Centrifuging whole blood spins the formed elements to the bottom of the tube, with plasma forming the top layer (see Figure 29.1). Since the blood cell population is primarily RBCs, the hematocrit is generally considered equivalent to the RBC volume, and this is the only value reported. However, the relative percentage of WBCs can be differentiated, and both WBC and plasma volume will be reported here. Normal hematocrit values for the male and female, respectively, are 47.0 ± 5 and 42.0 ± 5 .



Prepare for lab: Watch the Pre-Lab Video

Mastering A&P*>Study Area > Pre-Lab Videos

Activity 4

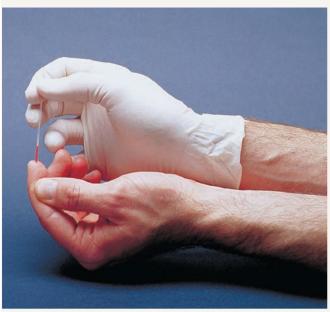
Determining the Hematocrit

The hematocrit is determined by the micromethod, so only a drop of blood is needed. If possible (and the centrifuge allows), all members of the class should prepare their capillary tubes at the same time so the centrifuge can be run only once.

- 1. Obtain two heparinized capillary tubes, capillary tube sealer or modeling clay, a lancet, alcohol swabs, and some cotton balls.
- 2. If you are using your own blood, use an alcohol swab to cleanse a finger, prick the finger with a lancet, and allow the blood to flow freely. Wipe away the first few drops and, holding the red-line-marked end of the capillary tube to the blood drop, allow the tube to fill at least three-fourths full by capillary action (**Figure 29.6a**). If the blood is not flowing freely, the end of the capillary tube will not be completely submerged in the blood during filling, air will enter, and you will have to prepare another sample.

If you are using instructor-provided blood, simply immerse the red-marked end of the capillary tube in the blood sample and fill it three-quarters full as just described.

- 3. Plug the blood-containing end by pressing it into the capillary tube sealer or clay (Figure 29.6b). Prepare a second tube in the same manner.
- 4. Place the prepared tubes opposite one another in the radial grooves of the microhematocrit centrifuge with the sealed ends abutting the rubber gasket at the centrifuge periphery (Figure 29.6c). This loading procedure balances the centrifuge and prevents blood from spraying everywhere by centrifugal force. *Make a note of the numbers of the grooves your tubes are in.* When all the tubes have been loaded, make sure the centrifuge is properly balanced, and secure the centrifuge cover. Turn the centrifuge on, and set the timer for 4 or 5 minutes.







(c)

Figure 29.6 Steps in a hematocrit determination. (a) Fill a heparinized capillary tube with blood. (b) Plug the blood-containing end of the tube with clay.

(c) Place the tube in a microhematocrit centrifuge. (Centrifuge must be balanced.)

(b)

5. Determine the percentage of RBCs, WBCs, and plasma by using the microhematocrit reader. The RBCs are the bottom layer, the plasma is the top layer, and the WBCs are the buff-colored layer between the two. If the reader is not available, use a millimeter ruler to measure the length of the filled capillary tube occupied by each element, and compute its percentage by using the following formula:

 $\frac{\text{Height of the column composed of the element (mm)}}{\text{Height of the original column of whole blood (mm)}} \times 100$

Record your calculations below and on the data sheet (p. 438).

% RBC ______ % WBC _____ % plasma _____

Usually WBCs constitute 1% of the total blood volume. How do your blood values compare to this figure and to the normal percentages for RBCs and plasma? (See Figure 29.1.)

As a rule, a hematocrit is considered a more accurate test than the total RBC count for determining the RBC composition of the blood. A hematocrit within the normal range generally indicates a normal RBC number, whereas an abnormally high or low hematocrit is cause for concern.

Hemoglobin Concentration

As noted earlier, a person can be anemic even with a normal RBC count. Since hemoglobin (Hb) is the RBC protein responsible for oxygen transport, perhaps the most accurate way of measuring the oxygen-carrying capacity of the blood is to determine its hemoglobin content. Oxygen, which

combines reversibly with the heme (iron-containing portion) of the hemoglobin molecule, is picked up by the blood cells in the lungs and unloaded in the tissues. Thus, the more hemoglobin molecules the RBCs contain, the more oxygen they will be able to transport. Normal blood contains 12 to 18 g of hemoglobin per 100 ml of blood. Hemoglobin content in men is slightly higher (13 to 18 g) than in women (12 to 16 g).

Activity 5

Determining Hemoglobin Concentration

Several techniques have been developed to estimate the hemoglobin content of blood, ranging from the old, rather inaccurate Tallquist method to expensive hemoglobinometers, which are precisely calibrated and yield highly accurate results. Directions for both the Tallquist method and a hemoglobinometer are provided here.

Tallquist Method

- 1. Obtain a Tallquist hemoglobin scale, test paper, lancets, alcohol swabs, and cotton balls.
- 2. Use instructor-provided blood or prepare the finger as previously described. (For best results, make sure the alcohol evaporates before puncturing your finger.) Place one good-sized drop of blood on the special absorbent paper provided with the color scale. The blood stain should be larger than the holes on the color scale.



(a) A drop of blood is added to the moat plate of the blood chamber. The blood must flow freely.



(c) The charged blood chamber is inserted into the slot on the side of the hemoglobinometer.

- 3. As soon as the blood has dried and loses its glossy appearance, match its color, under natural light, with the color standards by moving the specimen under the comparison scale so that the blood stain appears at all the various apertures. (The blood should not be allowed to dry to a brown color, as this will result in an inaccurate reading.) Because the colors on the scale represent 1% variations in hemoglobin content, it may be necessary to estimate the percentage if the color of your blood sample is intermediate between two color standards.
- 4. On the data sheet (p. 438) record your results as the percentage of hemoglobin concentration and as grams per 100 ml of blood.

Hemoglobinometer Determination

1. Obtain a hemoglobinometer, hemolysis applicator, alcohol swab, and lens paper, and bring them to your bench.



(b) The blood sample is hemolyzed with a wooden hemolysis applicator. Complete hemolysis requires 35 to 45 seconds.



(d) The colors of the green split screen are found by moving the slide with the right index finger. When the two colors match in density, the grams/100 ml and % Hb are read on the scale.

Figure 29.7 Hemoglobin determination using a hemoglobinometer.

Test the hemoglobinometer light source to make sure it is working; if not, request new batteries before proceeding and test it again.

- 2. Remove the blood chamber from the slot in the side of the hemoglobinometer and disassemble the blood chamber by separating the glass plates from the metal clip. Notice as you do this that the larger glass plate has an H-shaped depression cut into it that acts as a moat to hold the blood, whereas the smaller glass piece is flat and serves as a coverslip.
- 3. Clean the glass plates with an alcohol swab, and then wipe them dry with lens paper. Hold the plates by their sides to prevent smearing during the wiping process.
- 4. Reassemble the blood chamber (remember: larger glass piece on the bottom with the moat up), but leave the moat plate about halfway out to provide adequate exposed surface to charge it with blood.
- 5. Obtain a drop of blood (from the provided sample or from your fingertip as before), and place it on the depressed area of the moat plate that is closest to you (Figure 29.7a).
- 6. Using the wooden hemolysis applicator, stir or agitate the blood to rupture (lyse) the RBCs (Figure 29.7b). This usually takes 35 to 45 seconds. Hemolysis is complete when the blood appears transparent rather than cloudy.
- 7. Push the blood-containing glass plate all the way into the metal clip and then firmly insert the charged blood

- chamber back into the slot on the side of the instrument (Figure 29.7c).
- 8. Hold the hemoglobinometer in your left hand with your left thumb resting on the light switch located on the underside of the instrument. Look into the eyepiece and notice that there is a green area divided into two halves (a split field).
- 9. With the index finger of your right hand, slowly move the slide on the right side of the hemoglobinometer back and forth until the two halves of the green field match (Figure 29.7d).
- 10. Note and record on the data sheet (p. 438) the grams of Hb (hemoglobin)/100 ml of blood indicated on the uppermost scale by the index mark on the slide. Also record % Hb, indicated by one of the lower scales.
- 11. Disassemble the blood chamber once again, and carefully place its parts (glass plates and clip) into a bleachcontaining beaker.

Generally speaking, the relationship between the hematocrit and grams of hemoglobin per 100 ml of blood is 3:1for example, a hematocrit of 36% with 12 g of Hb per 100 ml of blood is a ratio of 3:1. How do your values compare?

Record on the data sheet (p. 438) the value obtained from vour data.

Bleeding Time

Normally a sharp prick of the finger or earlobe results in bleeding that lasts from 2 to 7 minutes (Ivy method) or 0 to 5 minutes (Duke method), although other factors such as altitude affect the time. How long the bleeding lasts is referred to as **bleeding time** and tests the ability of platelets to stop bleeding in capillaries and small vessels. Absence of some clotting factors may affect bleeding time, but prolonged bleeding time is most often associated with deficient or abnormal platelets.

Coagulation Time

Blood clotting, or **coagulation,** is a protective mechanism that minimizes blood loss when blood vessels are ruptured.

This process requires the interaction of many substances normally present in the plasma (clotting factors, or procoagulants) as well as some released by platelets and injured tissues. Basically hemostasis proceeds as follows (Figure 29.8a, p. 436): The injured tissues and platelets release **tissue factor** (TF) and PF₃ (platelet factor 3) respectively, which trigger the clotting mechanism, or cascade. Tissue factor and PF₃ interact with other blood protein clotting factors and calcium ions to form **prothrombin activator**, which in turn converts prothrombin (present in plasma) to thrombin. Thrombin then acts enzymatically to polymerize (combine) the soluble fibrinogen proteins (present in plasma) into insoluble fibrin, which forms a meshwork of strands that traps the RBCs and forms the basis of the clot (Figure 29.8b). Normally, blood removed from the body clots within 2 to 6 minutes.

Activity 6

Record the time. _

Determining Coagulation Time

- 1. Obtain a nonheparinized capillary tube, a timer (or watch), a lancet, cotton balls, a triangular file, and alcohol swabs.
- 2. Clean and prick the finger to produce a free flow of blood. Discard the lancet in the disposal container.
- 3. Place one end of the capillary tube in the blood drop, and hold the opposite end at a lower level to collect the sample.
- 4. Lay the capillary tube on a paper towel after collecting the sample.
- illary tubes into the sharps container.
- 6. Put used supplies in the autoclave bag and broken cap-

5. At 30-second intervals, make a small nick on the tube close

to one end with the triangular file, and then carefully break the tube. Slowly separate the ends to see whether a gel-like

thread of fibrin spans the gap. When this occurs, record be-

low and on the data sheet (p. 438) the time for coagulation to

occur. Are your results within the normal time range?

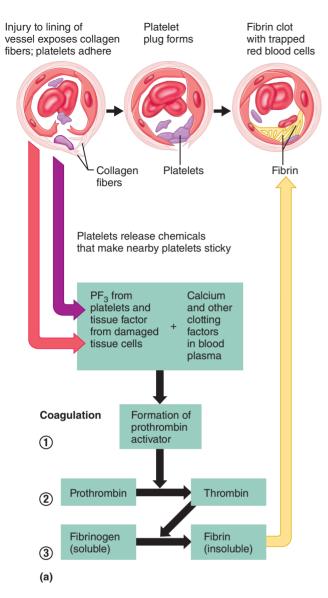




Figure 29.8 Events of hemostasis and blood clotting. (a) Simple schematic of events. Steps numbered 1–3 represent the major events of coagulation. (b) Photomicrograph of RBCs trapped in a fibrin mesh (2700×).

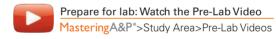
(b)

Blood Typing

Blood typing is a system of blood classification based on the presence of specific glycoproteins on the outer surface of the RBC plasma membrane. Such proteins are called **antigens**, or **agglutinogens**, and are genetically determined. For ABO blood groups, these antigens are accompanied by plasma proteins, called **antibodies** or **agglutinins**. These antibodies act against RBCs carrying antigens that are not present on the person's own RBCs. If the donor blood type doesn't match, the recipient's antibodies react with the donor's blood antigens, causing the RBCs to clump, agglutinate, and eventually hemolyze. It is because of this phenomenon that a person's blood must be carefully typed before a whole blood or packed cell transfusion.

Several blood typing systems exist, based on the various possible antigens, but the factors routinely typed for are antigens of the ABO and Rh blood groups which are most commonly involved in transfusion reactions. The basis of the ABO typing is shown in **Table 29.2**.

Individuals whose red blood cells carry the Rh antigen are Rh positive (approximately 85% of the U.S. population); those lacking the antigen are Rh negative. Unlike ABO blood groups, the blood of neither Rh-positive (Rh+) nor Rh-negative (Rh-) individuals carries preformed anti-Rh antibodies. This is understandable in the case of the Rh-positive individual. However, Rh-negative persons who receive transfusions of Rh-positive blood become sensitized by the Rh antigens of the donor RBCs, and their systems begin to produce anti-Rh antibodies. On subsequent exposures to Rh-positive blood, typical transfusion reactions occur, resulting in the clumping and hemolysis of the donor blood cells.



Activity 7

Typing for ABO and Rh Blood Groups

Blood may be typed on microscope slides or using blood test cards. Each method is described in this activity. The artificial blood kit does not use any body fluids and produces results similar to but not identical to results for human blood.

Typing Blood Using Glass Slides

- 1. Obtain two clean microscope slides, a wax marking pencil, anti-A, anti-B, and anti-Rh typing sera, toothpicks, lancets, alcohol swabs, medicine dropper, and the Rh typing box.
- 2. Divide slide 1 into halves with the wax marking pencil. Label the lower left-hand corner "anti-A" and the lower right-hand corner "anti-B." Mark the bottom of slide 2 "anti-Rh."
- 3. Place one drop of anti-A serum on the *left* side of slide 1. Place one drop of anti-B serum on the *right* side of slide 1. Place one drop of anti-Rh serum in the center of slide 2.
- 4. If you are using your own blood, cleanse your finger with an alcohol swab, pierce the finger with a lancet, and wipe away the first drop of blood. Obtain 3 drops of freely flowing blood, placing one drop on each side of slide 1 and a drop on slide 2. Immediately dispose of the lancet in a designated disposal container.

Table 29.2 A	BO Blood Typing				
			% o	f U.S. popula	ation
ABO blood type	Antigens present on RBC membranes	Antibodies present in plasma	White	Black	Asian
A	A	Anti-B	40	27	28
В	В	Anti-A	11	20	27
AB	A and B	None	4	4	5
0	Neither	Anti-A and anti-B	45	49	40

If using instructor-provided animal blood or red blood cells treated with EDTA (an anticoagulant), use a medicine dropper to place one drop of blood on each side of slide 1 and a drop of blood on slide 2.

- 5. Quickly mix each blood-antiserum sample with a fresh toothpick. Then dispose of the toothpicks and used alcohol swab in the autoclave bag.
- 6. Place slide 2 on the Rh typing box and rock gently back and forth. (A slightly higher temperature is required for precise Rh typing than for ABO typing.)
- 7. After 2 minutes, observe all three blood samples for evidence of clumping. The agglutination that occurs in the positive test for the Rh factor is very fine and difficult to interpret; thus if there is any question, observe the slide under the microscope. Record your observations in the **Activity 7 chart.**
- 8. Interpret your ABO results (see the examples of each type) in Figure 29.9. If clumping was observed on slide 2, you are Rh positive. If not, you are Rh negative.
- 9. Record your blood type on the data sheet (p. 438).
- 10. Put the used slides in the bleach-containing bucket at the general supply area; put disposable supplies in the autoclave bag.

Activity 7: Blood Typing		
Result	Observed (+)	Not observed (-)
Presence of clumping with anti-A		
Presence of clumping with anti-B		
Presence of clumping with anti-Rh		

Using Blood Typing Cards

- 1. Obtain a blood typing card marked A, B, and Rh, dropper bottles of anti-A serum, anti-B serum, and anti-Rh serum, toothpicks, lancets, and alcohol swabs.
- 2. Place a drop of anti-A serum in the spot marked anti-A, place a drop of anti-B serum on the spot marked anti-B, and place a drop of anti-Rh serum on the spot marked anti-Rh (or anti-D).

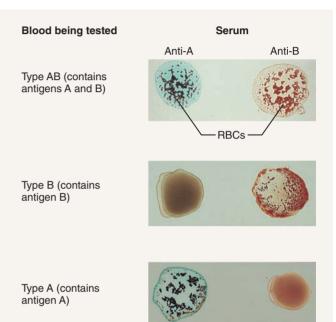


Figure 29.9 Blood typing of ABO blood types.

Type O (contains no antigen)

When serum containing anti-A or anti-B antibodies (agglutinins) is added to a blood sample, agglutination will occur between the antibody and the corresponding antigen (agglutinogen A or B). As illustrated, agglutination occurs with both sera in blood group AB, with anti-B serum in blood group B, with anti-A serum in blood group A, and with neither serum in blood group O.

- 3. Carefully add a drop of blood to each of the spots marked "Blood" on the card. If you are using your own blood, refer to step 4 in the Activity 7 section Typing Blood Using Glass Slides. Immediately discard the lancet in the designated disposal container.
- 4. Using a new toothpick for each test, mix the blood sample with the antibody. Dispose of the toothpicks appropriately.

- 5. Gently rock the card to allow the blood and antibodies to mix.
- 6. After 2 minutes, observe the card for evidence of clumping. The Rh clumping is very fine and may be difficult to observe. Record your observations in the Activity 7: Blood Typing chart. (Use Figure 29.9 to interpret your results.)
- 7. Record your blood type on the data sheet below, and discard the card in an autoclave bag.

Activity 8

Observing Demonstration Slides

Look at the slides of macrocytic hypochromic anemia, microcytic hypochromic anemia, sickle cell anemia, lymphocytic leukemia (chronic), and eosinophilia that have been put on demonstration by your instructor. Record your observations in the appropriate section of the Review Sheet. You can refer to your notes, the text, and other references later to respond to questions about the blood pathologies represented on the slides.

Cholesterol Concentration in Plasma

Atherosclerosis is the disease process in which the body's blood vessels become increasingly occluded, or blocked, by plaques. By narrowing the arteries, the plaques can contribute to hypertensive heart disease. They also serve as starting points for the formation of blood clots (thrombi), which may break away and block smaller vessels farther downstream in the circulatory pathway and cause heart attacks or strokes.

Cholesterol is a major component of the smooth muscle plaques formed during atherosclerosis. No physical examination of an adult is considered complete until cholesterol levels are assessed along with other risk factors. A normal value for total plasma cholesterol in adults ranges from 130 to 200 mg per 100 ml of plasma; you will use blood to make such a determination.

Although the total plasma cholesterol concentration is valuable information, it may be misleading, particularly if a person's high-density lipoprotein (HDL) level is high and low-density lipoprotein (LDL) level is relatively low. Cholesterol, being water insoluble, is transported in the blood complexed to lipoproteins. In general, cholesterol bound into HDLs is destined to be degraded by the liver and then eliminated from the body, whereas that forming part of the LDLs is "traveling" to the body's tissue cells. When LDL levels are excessive, cholesterol is deposited in the blood vessel walls; hence, LDLs are considered to carry the "bad" cholesterol.

Activity 9

Measuring Plasma Cholesterol Concentration

- 1. Go to the appropriate supply area, and obtain a cholesterol test card and color scale, lancet, and alcohol swab.
- 2. Clean your fingertip with the alcohol swab, allow it to dry, then prick it with a lancet. Place a drop of blood on the test area of the card. Put the lancet in the designated disposal container.
- 3. After 3 minutes, remove the blood sample strip from the card and discard in the autoclave bag.
- 4. Analyze the underlying test spot, using the included color scale. Record the cholesterol level below and on the Hematologic Test Data Sheet.

Total cholesterol	level	ma/d
TOTAL CHOICSTELD	10001	IIIu/u

5. Before leaving the laboratory, use the spray bottle of bleach solution, and saturate a paper towel to thoroughly wash down your laboratory bench.

Hematologic Test Data Sheet					
Differential WBC count:			Hemoglobin (Hb) content:		
WBC	Student blood smear	Unknown sample #	Tallquist method: g/100 ml of blood; % Hb		
% neutrophils			Hemoglobinometer (type:		
% eosinophils)		
% basophils			g/100 ml of blood; %Hb		
% lymphocytes			Ratio (hematocrit to grams of Hb per 100 ml of blood):		
% monocytes					
Hematocrit:			Coagulation time:		
RBC % of blood volume Blo			Blood typing:		
WBC % of blood volume not generally reported			ABO group Rh factor		
Plasma % of blood		· sportou	Total cholesterol level: mg/dl of blood		

Name		LabTime/Date					
Composition of Bloc	od						
 What is the blood volume of an average-size adult male? liters; an average adult female? What determines whether blood is bright red or a dull brick red? 							
						3. Use the key to identify the cowill be used more than once.	
Key: a. red blood cell	d. basophil	g. lymphocyte					
b. megakaryocytec. eosinophil	e. monocyte f. neutrophil	h. formed element	S				
c. eosinopini	i. neutropini	i. plasma					
1. most num	nerous leukocyte						
	,, and 2. granulocytes (3)						
3. also calle	3. also called an erythrocyte; anucleate formed element						
	,, 4. phagocytic leukocytes (3)						
	, 5. agranulocytes						
6. precursor	6. precursor cell of platelets						
7. (a) throug	7. (a) through (g) are all examples of these						
8. involved	8. involved in destroying parasitic worms						
9. releases h	9. releases histamine; promotes inflammation						
10. produces	10. produces antibodies						
11. transport	11. transports oxygen						
12. primarily	12. primarily water, noncellular; the fluid matrix of blood						
13. exits a blo	ood vessel to develop into a mad	rophage					
	,, 14. the five types of white blo	ood cells					

4.		, ,	
			, and nd
	Name three ions		, and
5.	Describe the consist	ency and color of the plasma you observed in tl	ne laboratory
6.	What is the average	life span of a red blood cell? How does its anuc	leate condition affect this life span?
7.	sible, and note the p	ribe the structural characteristics of each of the percentage of each in the total white blood cell p	
	neutrophils:		
	lymphocytes:		
	basophils:		
	monocytes:		
8.	Correctly identify the	e blood pathologies described in column A by m	natching them with selections from column B:
	Column A		Column B
		_ 1. abnormal increase in the number of WBCs	a. anemia
		_ 2. abnormal increase in the number of RBCs	b. leukocytosis
		_ 3. condition of too few RBCs or of RBCs with hemoglobin deficiencies	c. leukopenia
		_ 4. abnormal decrease in the number of WBCs	d. polycythemia

Hematologic Tests				
9. Broadly speaking, why are	e hematologic stud	dies of blood so important in	diagnosing disease	9?
10. In the chart below, record recording values for healt		n the blood tests you read a d indicating the significance o		
	Student	Normal values	Si	ignificance
Test	test results	(healthy male adults)	High values	Low values
Total WBC count	No data			
Total RBC count	No data			
Hematocrit				
Hemoglobin determination				
Bleeding time	No data			
Coagulation time				
11. Why is a differential WBC o	count more valuab	le than a total WBC count wh	en trying to determ	ine the specific source o
pathology?				
12. What name is given to the	process of RBC p	roduction? (Consult an appro	opriate reference as	necessary.)
What hormone acts as a s	timulus for this pr	ocess?		
Why might patients with k	idney disease suf	fer from anemia?		
How can such patients be	treated?			
Discuss the effect of each explain your reasoning.	of the following fa	actors on RBC count. Consult	an appropriate refe	erence as necessary, an
long-term effect of athletic	c training (for exar	mple, running 4 to 5 miles pe	r day over a period	of 6 to 9 months):

Blood drop and anti-A serum

Blood drop and anti-B serum





Blood drop and anti-A serum



Blood drop and anti-B serum

On the basis of these results, Mr. Adams has type _______ blood, and Mr. Calhoon has type ______ blood.

20.		Explain why an Rh-negative person does not have a transfusion reaction on the first exposure to Rh-positive blood but <i>does</i> have a reaction on the second exposure.					
	Wh	at happens when an ABO blood type is mismatched for the first time?					
21.	Red	Record your observations of the five demonstration slides viewed.					
	a.	Macrocytic hypochromic anemia:					
	b.	Microcytic hypochromic anemia:					
	c.	Sickle cell anemia:					
	d. Lymphocytic leukemia (chronic):						
	e.	Eosinophilia:					
	Wh	ich of the slides above (a through e) corresponds with the following conditions?					
		1. iron-deficient diet					
		2. a type of bone marrow cancer					
		3. genetic defect that causes hemoglobin to become sharp/spiky					
		4. lack of vitamin B ₁₂					
		5. a tapeworm infestation in the body					
		6. a bleeding ulcer					
22.	Pro	vide the normal, or at least "desirable," range for plasma cholesterol concentration.					
		mg/100 ml					
23.	 Describe the relationship between high blood cholesterol levels and cardiovascular diseases such as hypertensic heart attacks, and strokes. 						