I. Objectives

A. To identify blood cell types using the models.

B. To examine a blood smear and identify its various formed elements.

C. To determine the amount of hemoglobin in a blood sample.

D. To determine the percentage of formed elements in a blood sample.

E. To determine blood type as to ABO grouping and Rh factor.

II. The Blood

Blood is a specialized connective tissue that circulates throughout the body in a system of tubes. It has many functions. Among them are the transport of wastes, nutrients, hormones, heat, and cells and molecules to combat infection. Blood is a tissue that can be easily obtained and is widely used to provide clinicians with information about the status of the human body.

Blood is composed of liquid, or plasma, and formed elements (cells or fragments of cells). Plasma is a complex solution of ions and molecules in water. The formed elements include the erythrocytes (red blood cells), leukocytes (white blood cells), and platelets (fragments of a larger cell found in the bone marrow called a megakaryocyte). There are five different kinds of leukocytes placed into two different groups. The first group consists of the granulocytes. They include three types of white blood cells: neutrophils, basophils, and eosinophils. The second group is composed of the agranulocytes (so called because they lack cytoplasmic granules). They include two types: lymphocytes and monocytes.
A. Examination of Blood Cell Models and a Blood Smear

1. Blood Models. Using the blood models, please identify and draw the types of blood cells.

2. Human Blood Slide. For this exercise you will examine a prepared slide of human blood. This slide was made by placing a thin smear of blood on a clean microscope slide and then staining so the various blood cells can be viewed through a light microscope. The stain most commonly used for this purpose is Wright’s stain. It contains eosin (an acid dye) and methylene blue (a basic dye). Organelles within the leukocytes take up the dyes differently and this helps in the identification of the specific types of white blood cells. If a differential white cell count is to be made, usually 100 white cells are counted and the percentages are recorded for each type. This aids the clinician in the diagnosis and treatment of various diseases. For example, mononucleosis is characterized by a decrease in the percentage of granulocytes and an increase in the percentage of agranulocytes, and an elevated total number of white cells.
Examine the blood smear under the 10× objective of the microscope and then under the 40× objective. Make a drawing of what you see under each objective and label your drawing indicating the following structures.

The numerous red blood cells will appear pink since they take up the dye eosin. The nuclei of the white blood cells will be stained blue-purple. Examine various areas of the slide. Which cell type (red or white) is more numerous? Now examine and draw a picture of the blood smear under oil immersion set up by your instructor on the side counter. Examine various white blood cells. By using your textbook as a guide can you find a neutrophil and draw a picture of it in the box below? They make up about 60% of the leukocyte population and are easily identified by the nucleus which is segmented into 2–5 lobes. Cytoplasmic granules may not be obvious as these cells react only weakly with the dyes (they are “neutral”). Can you locate a lymphocyte and draw a picture of it in the box below? They make up 25–30% of the population of white blood cells and come in three sizes. They have a large round nucleus which stains dark. The other white cells may be hard to locate as they are not numerous. Demonstration slides may be set up to show them.
Examine the red blood cells. Do you see a nucleus? The red cells are actually **biconcave** as shown in your textbook. Do they look like the illustrations in your text? If you look closely you should be able to find some platelets. They are very small and usually stain blue or purple. Please draw red blood cells and platelets in the box below.

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**Red Blood Cells and Platelets**

Examine a slide of **leukemic** blood. How does this slide differ from the slide of normal blood? See your textbook. Examine a slide of **sickle cell** blood. Locate some sickled cells. See your textbook. How common is this condition in the United States population? Please draw a picture of leukemic and sickle cell blood in the boxes below.
B. Determination of the Amount of Hemoglobin in Blood

NOTE: Whether working with human blood or any other animal blood, it is a good precaution to wear gloves whenever you work with blood.

The major function of hemoglobin is to carry oxygen and assist in the transport of carbon dioxide in the bloodstream. Its concentration is therefore an important indicator of the amount of oxygen that can be delivered to cells. Normal blood contains about 14 grams of hemoglobin in each 100 mL of blood, but this can vary depending upon age and sex.

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Amount of Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult males</td>
<td>13–18 g./dL.</td>
</tr>
<tr>
<td>Adult females</td>
<td>11–14 g./dL.</td>
</tr>
<tr>
<td>Infants</td>
<td>10–14 g./dL.</td>
</tr>
<tr>
<td>Children</td>
<td>Gradual increase from infant to adult levels</td>
</tr>
</tbody>
</table>

Each gram of hemoglobin can combine with about 1.34 mL of oxygen. So normal blood can carry approximately 19 mL of oxygen per 100 mL of blood.

Hemoglobin is the oxygen-carrying pigment and main component of red blood cells. Low hemoglobin levels may indicate anemia, recent hemorrhage, or fluid retention. Elevated hemoglobin levels may indicate polycythemia or dehydration.

There are a number of methods that can be used to measure the amount of hemoglobin in a given quantity of blood. In our exercise we will use one of the more updated methods known as the STAT-Site M<sup>High</sup> Test. This method provides a direct reading of hemoglobin concentration. The test consists of a plastic card with reagent pads for determining the concentration of hemoglobin. When a drop of whole blood is applied to the top of the STAT-Site M<sup>High</sup> Test Card, hemolysis occurs and hemoglobin is released. There is sodium nitrite in the pad that converts the hemoglobin to methemoglobin. Sodium azide (also in the pad) reacts with methemoglobin to form azide-methemoglobin which is brown and can be detected using the meter provided. The amount of color produced is proportional to the concentration of hemoglobin in the sample.
3. Code the Meter

**NOTE:** Before you begin and after you finish please wipe down the meter with an alcohol pad. Also, **be sure to wear gloves at all times.**

Press the on button to turn on the power. To conserve energy, the meter will automatically shut off if left idle for more than 2 minutes. If the Code number on the display matches the Code number on the Test Card, go to Step 2. If it does not, please inform your instructor.

4. Insert the Test Card

The flashing Test Card symbol indicates that you should insert the Test Card. Insert a Test Card with a Code number that matches the Code displayed on the screen at power on. Slide the edges of the test card under the Guide tabs on the Test Card holder. Be sure to insert the card all the way to the back. You should feel it lock into place.

When the display shows the Test Type (Hgb), an unblinking Test Card symbol and a Flashing Drop symbol, it is time to apply the sample.

![Image of a test meter with a code card inserted and a test card symbol indicating measurement of hemoglobin](Figure 14-1. MEASURING THE AMOUNT OF HEMOGLOBIN)
**NOTE:** When performing a fingerstick, washing your hands under warm water can increase blood flow.

To perform this test you will need one drop of whole blood. Using the lancet provided, make a small skin puncture. The puncture site should be cleaned with an alcohol swab and allowed to dry. Wipe away the first drop of blood with a gauze pad and allow a large drop to form at the puncture site. Do not “milk” the finger. Position the drop of blood directly over the center of the Test Card and carefully lay the drop of blood on the center of the card. After applying the sample to the center of the Test Card, the countdown to test result will begin. The test may finish before reaching zero. The test provides a direct reading of hemoglobin concentration in whole blood between 6 and 21 g./dL. Values above or below this will display as <Lo> or <High>. Record your result in the space below and remove the Test Card. To do this, lift slightly as you slide the Test Card out of the meter. Dispose of the Test Card properly.

**CAUTION:** As with all chemical reagents, contact with the skin should be avoided with the reactive areas of the Card. Always handle blood specimens as potentially infectious samples.

5. **Record these results below.**

   a. Hgb value _________ g./dL.

   b. Using these results (and knowing that there is 1.34 mL. of oxygen per gram of hemoglobin) calculate how much oxygen 100 mL. of this blood can carry. _________

   c. What might it indicate if the hemoglobin value is too low?

   What might it indicate if the hemoglobin value is too high?

   d. What is anemia? ________________
C. Hematocrit

The hematocrit is the percentage of formed elements in the blood. It is determined by centrifuging a sample of blood to separate the plasma from the formed elements. Since more than 99% of the formed elements are red blood cells, the hematocrit is commonly assumed to be the percentage of red cells in the blood sample tested. This test is easy to perform and is possibly a more accurate estimate of red cell concentration than is the red blood cell count (where the number of red blood cells per volume of blood is estimated). A human hematocrit averages about 45%.

Once again, wearing gloves and using sheep blood, insert one end of a heparinized capillary tube into the blood drop and let it fill about ¾ full by capillary action. To do this hold the tube horizontally or tilted slightly downward. Now apply your index finger to the end of the tube and press the blood-containing end into a tray of Critoseal to plug it.

Place the tube in a groove in the centrifuge head of a microhematocrit centrifuge with the sealed end toward the periphery of the head. Record the number of the groove used if other blood samples are being centrifuged at the same time. Your instructor will secure the lid on the head and centrifuge the tube(s) for 5 minutes. After 5 minutes remove your tube and read it in the hematocrit reader. The tube should resemble the one shown in your textbook.

What was the hematocrit? ________________________________________

What is polycythemia? ____________________________________________

D. Blood Typing

Human blood is grouped as O, A, B, or AB. These groupings are based on the presence or absence of antigens, or agglutinogens, on the membranes of the red blood cells. If the red cells have the A antigen, the blood type is A. Both A and B antigens are present on cells of blood type AB. Blood type O has no antigens on the red cell membrane and type B has B antigens.

The antigens react with antibodies, or agglutinins, which are found in the plasma. Anti-A antibody would cause the red blood cells of blood type A or AB to agglutinate or clump. Anti-B agglutinin would do the same with blood of types B or AB. Whichever antigen is found on the red cells, its reciprocal antibody is found in the plasma. By the use of serum (plasma minus its fibrinogen) that contains antibodies, we can easily type a given blood sample.

Using the blood simulation kit provided, your instructor will demonstrate how blood is actually typed. Follow the procedure outlined below.
1. Procedure One

   a. Using a clean blood typing plate with 3 depressions (one for A, B, and one for Rh factor). Place one drop of Anti-A serum (containing antibodies against A antigens) in one of the depressions, and a drop of Anti-B in a second depression.

   b. Next place a drop of simulated blood in each drop of serum. Mix the samples with different toothpicks. Observe each sample for clumping of the blood. If the blood mixed with the Anti-A serum clumps, the blood is type A. What type is it if it clumps in both samples? Neither?

Repeat this procedure for each of the four blood samples and record your results below (What was the blood type in each case?):

   i. Sample 1, Mr. Smith ________________________________
   ii. Sample 2, Mr. Green ________________________________
   iii. Sample 3, Mr. Brown ________________________________
   iv. Sample 4, Mr. Jones ________________________________

   c. Your instructor may also ask you to type these samples for the Rh factor. Rh+ blood contains an antigen that will cause cell clumping when exposed to Rh antibody. Rh– blood contains no Rh antigen on the red cell membrane, and no Rh antibody in the plasma unless the Rh– person has been previously given Rh+ blood. In this way the Rh system differs from the ABO system; that is the Rh antibody does not normally exist in the plasma of an Rh– person unless they have been previously given the blood containing the antigen. In the ABO system the reciprocal antibody is always present in the plasma.

The Rh system actually contains 12 different antigens. Antigen D is the most antigenic of them and is the one referred to when the Rh factor is discussed.

2. Procedure Two

   a. In the third depression (marked Rh) of the blood typing plates you used above, place a drop of Anti-D serum (contains Rh antibodies) in its center. Add a drop of simulated blood and stir with a toothpick.

   b. Place the slide on a typing box and gently mix with toothpick. Examine the sample for clumping. Clumping would indicate that the blood is Rh+. Results:
Repeat this procedure for each of the four blood samples and record your results below (What was the blood type in each sample with reference to the Rh factor?):

i. Sample 1, Mr. Smith ________________________________

ii. Sample 2, Mr. Green ________________________________

iii. Sample 3, Mr. Brown ________________________________

iv. Sample 4, Mr. Jones ________________________________

c. Which of the following blood types is the most common? The most rare? The “universal donor?” The “universal recipient?”

   i. A+
   ii. A−
   iii. B+
   iv. B−
   v. AB+
   vi. AB−
   vii. O+
   viii. O−

d. What would happen if a pint of O+ blood were given to a person with blood type B−? If the person had received O+ blood before would your answer be different?
Fill in the table below.*

<table>
<thead>
<tr>
<th>Table 14-1. BLOOD TYPES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Type</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>AB</td>
</tr>
<tr>
<td>O</td>
</tr>
</tbody>
</table>

Determine which type of blood each of the following subjects may receive, or to whom they can donate blood. Also be sure you know which blood type is the universal donor and which blood type is the universal recipient.

<table>
<thead>
<tr>
<th>Table 14-2. BLOOD DONORS AND RECIPIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can Donate To:</td>
</tr>
<tr>
<td>AB−</td>
</tr>
<tr>
<td>AB+</td>
</tr>
<tr>
<td>O−</td>
</tr>
<tr>
<td>O+</td>
</tr>
<tr>
<td>A+</td>
</tr>
<tr>
<td>A−</td>
</tr>
<tr>
<td>B+</td>
</tr>
</tbody>
</table>

*As explained in Part B of this exercise.