WARD'S

Simulated Blood Smearing Lab Activity
Student Study Guide

BACKGROUND

DID YOU KNOW?
Prior to the mid-1500s, no one had any conception of blood circulating through the organs in the body.

Blood consists of both a liquid component, called plasma, and a non-liquid portion, which has many structures that are collectively called formed elements. Plasma is approximately 91% water; the remaining portion is made up of various albumins, fibrinogens, and globulins. In addition, the plasma contains food materials and gases such as oxygen, nitrogen, and carbon dioxide.

The formed elements include erythrocytes, or red blood cells (RBCs); various types of leukocytes, or white blood cells (WBCs); and platelets.

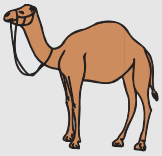
Blood cell type	Average per mm^3	Approximate diameter (microns)
Erythrocytes:		
Men	$4.5 – 6.0 \times 10^6$	5.5 – 8.8
Women	$4.3 – 5.5 \times 10^6$	
Leukocytes (total)	5,000 – 10,000	9 – 25
Granulocytes:		
Neutrophils	3,000 – 7,000	12 – 14
Eosinophils	50 – 400	9
Basophils	0 - 50	12
Agranulocytes:		
Monocytes	100 – 600	20 – 25
Lymphocytes	1,000 – 3,000	9 – 14
Platelets	250,000 – 400,000	2.5

DID YOU KNOW?
The average life span of a red blood cell is about 120 days.

Erythrocytes, or red blood cells, are circular, biconcave disks of 5-8 micrometers. Mature erythrocytes lack nuclei; they are lost in the course of development. Their chief function is to transport O_2 and CO_2. The transport of O_2 and CO_2 depends largely on the hemoglobin present in the erythrocytes. The biconcave shape is also related to the erythrocytes' function of transporting gases, in that it provides an increased surface area through which gases can diffuse.

The number of circulating RBCs is closely related to the blood's oxygen-carrying capacity. Any changes in the RBC count may be significant. RBC counts are routinely made to diagnose and evaluate the course of various diseases.

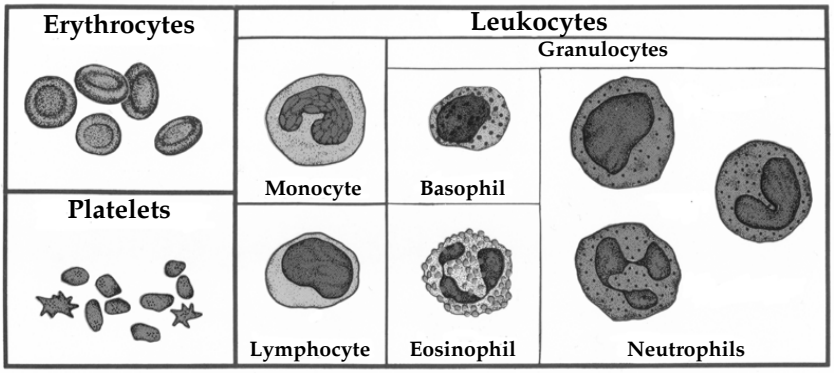
Human blood contains five types of white blood cells (leukocytes): lymphocytes, monocytes, neutrophils, eosinophils, and basophils.



DID YOU KNOW?
Camels and their relatives are the only mammals having oval red blood cells.

Phlebotomists collect blood samples and body fluids from patients for laboratory testing.

Blood Cell Types



Leukocytes function primarily to control various disease conditions, and WBC counts vary from 5,000 to 10,000 per mm^3. Certain infectious diseases are accompanied by an increase in WBC. If the number exceeds 10,000/mm^3, the person has an acute infection. If it drops below 5,000/mm^3, the person may have a condition such as measles or chicken pox. For diagnostic purposes it is important to estimate the relative count or percentage of each type of leukocyte in the blood.

This is done by a procedure called a differential count. The percentage of the different types of leukocytes present in the blood may change in particular diseases, and a differential count is important in their diagnosis. Unlike erythrocytes, leukocytes all have nuclei, but no hemoglobin. Leukocytes can move against the current of the bloodstream by amoeboid movement, and even pass through the blood vessel wall and enter the tissues.

Lymphocytes and monocytes (agranulocytes) are produced in the spleen, thymus, and lymph nodes. The neutrophils, eosinophils, and basophils (granulocytes) are produced in the bone marrow. Monocytes are the largest white cells that move actively by amoeboid motion and are able to ingest bacteria by engulfing (phagocytizing) them. They are of great importance in countering long-term infections. Neutrophils are similar to monocytes in that they phagocytize bacteria and are of prime importance in resisting acute bacterial infections. The number of neutrophils usually increases during bacterial infections. Lymphocytes are important in the body's specific immune responses, including antibody production. The majority of lymphocytes are found lodged in the lymphoid tissues; only a few are found in the blood. Eosinophils are also similar in function to the monocytes and neutrophils, and tend to increase greatly in number during allergic reactions and during parasitic infections. Basophils are active in phagocytosis, but their exact function is still largely unknown. They contain a number of biochemicals, such as histamine and heparin. Heparin prevents blood coagulation when histamine increases blood vessel permeability during inflammation.

DID YOU KNOW?
Humans have an average of 5.2 million erythrocytes per mm^3.

Platelets are small cytoplasmic fragments involved in the complex process of blood clotting. They are found in the red bone marrow as pinched-off portions of large cells called megakaryotes.

One of the most important examinations made in a clinical laboratory is a blood smear. Blood smears may be prepared by using either the glass slide or coverslip method. Although the coverslip method is considered more reliable, for practical reasons it is not widely used. In either method, a smear is prepared by spreading a drop of blood on the slide or coverslip, allowing it to dry, and then staining. The blood's cellular components can then be examined and counted under the microscope. A blood smear can provide essential information for diseases such as leukemia, sickle cell anemia, and malaria.

OBJECTIVES

- Understand the differences and functions of various blood components
- Prepare a blood smear, using two different methods.
- Examine and differentiate various blood components microscopically
- Perform differential cell counts of blood components

MATERIALS

Forensic serologists study blood, blood groups, and other bodily fluids for identification purposes following a crime.

MATERIALS NEEDED PER GROUP

Part A: Preparation of a Blood Smear

3 Glass microscope slides
2 Coverslips
1 Pipet
WARD'S Simulated Blood
Microscope (400X magnification)

Part B: Differential Blood Cell Counting

1 Ruled microscope slide
1 Coverslip
1 Pipet
WARD'S Simulated Blood
Microscope (400X magnification)

PROCEDURE

This simulated blood smearing activity does not use real blood or blood sera of any kind. WARD'S Simulated Blood contains microcomponents that simulate platelets and red and white blood cells. These microcomponents are similar in proportion to that of human blood, and can be seen under a microscope without staining. The red microcomponents represent erythrocytes, the blue represent lymphocytes, the yellow represent neutrophils, and the gray/black represent platelets.

Part A: Preparation of a Blood Smear

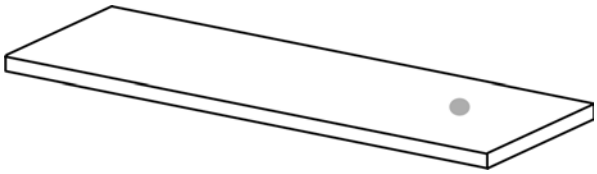
Glass Slide Method

DID YOU KNOW?
Sharks are able to smell a drop of blood from 1/4 mile away!

1. Thoroughly shake the vial of WARD'S Simulated Blood.

2. Lay a microscope slide on a flat surface and place a drop of WARD'S Simulated Blood approximately 1/2" from the back edge (Figure 1). Hold the slide at the front edge.

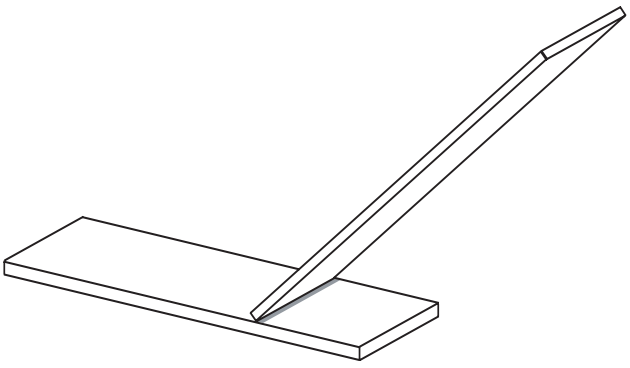
Figure 1



3. While holding the second "spreader" slide with thumb and index finger, bring it to rest at a 35-40° angle in front of the drop of blood, but not yet touching it.

4. Gently draw the spreader slide backward about halfway through the drop of blood, and allow the blood to spread across the edge of the spreader slide (Figure 2).

Figure 2



5. With a smooth, steady motion, slide the spreader across the slide. Use a gliding motion to avoid exerting a downward pressure (Figure 3). This will cause the Simulated Blood to smear in a gradual transition from thick to thin (Figure 4).

Figure 3

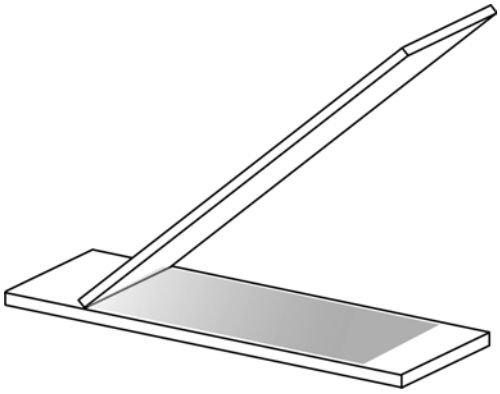
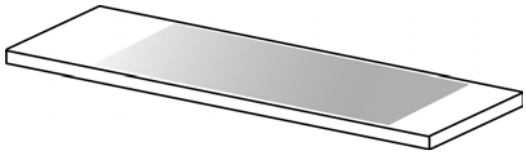


Figure 4



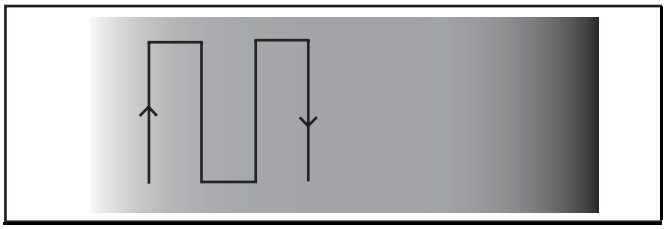
DID YOU KNOW?
Human blood travels a distance of 60,000 miles as it winds through arteries, arterioles, capillaries and back through the venules and veins.

6. The smear is now ready to be examined under the microscope, since staining is not required with WARD'S Simulated Blood. View the thin end of the smear under a microscope and use the examining pattern indicated in Figure 5.

NOTE

Blood smear characteristics are affected by the technique used. WARD'S Simulated Blood is specially formulated to mimic human blood regarding blood cell dispersal characteristics. Too steep of an angle on the spreader slide will result in a short smear. Good technique is essential in producing an acceptable smear.

Figure 5

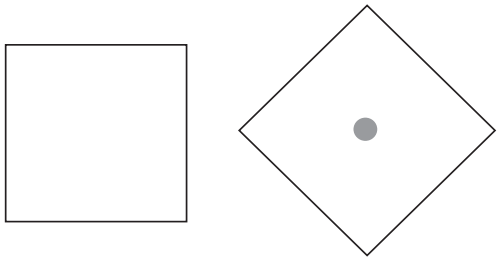


Cover Glass Method

1. Thoroughly shake the vial of WARD'S Simulated Blood.

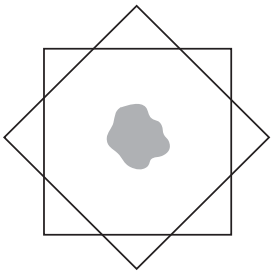
2. Place a drop of simulated blood in the middle of a cover glass (Figure 6).

Figure 6



3. Place another cover glass over the first slide as outlined in Figure 7. The simulated blood will spread quickly and evenly between the two cover glasses.

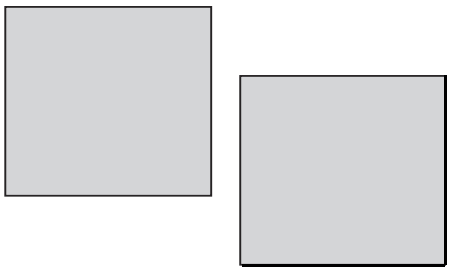
Figure 7



4. As soon as the blood has stopped spreading, quickly pull the cover glasses apart by holding them by their extended edges and sliding them horizontally in opposite directions (Figure 8).

The cover glasses should be pulled apart by sliding and not by lifting.

Figure 8



5. Mount the blood smears on glass microscope slides before examining under the microscope. Staining is not required since WARD'S Simulated Blood contains stained, nonbiological "cellular components."

DID YOU KNOW?

If you lined up all of the red blood cells in your body, they would be able to circle the equator and then some.

DID YOU KNOW?
Not all blood is red. It is also found in a variety of different colors including blue, green, and white.

Part B: Differential Blood Cell Counting

1. Thoroughly shake the vial of WARD'S Simulated Blood. Orient the slide so that the dull side of the grid is facing up and add a minute drop to the center grid of a ruled microscope slide. Carefully place a clean cover glass over the drop, trying not to trap any air bubbles. Allow the slide to sit undisturbed for approximately 5 minutes.

2. Place the slide on a microscope and examine at 10X. Locate a representative square on the ruled microscope slide and then switch to 40X.

3. Count four random fields of view within the square. Count the erythrocytes first, then the leukocytes (lymphocytes and neutrophils), and then the platelets. Record your counts for each field in Table 1 in the Analysis section of the lab.

Counting multiple fields of view results in greater accuracy. If time permits, count four fields of view from several other squares on the ruled microscope slide and calculate the average of these results.

4. Add your results for each component from all four fields of view and enter the total number of cells in Table 1.

5. Each square is 2 mm x 2 mm; the cover glass is approximately 0.1 mm above the counting area. Each square, therefore, has a volume of 2 mm x 2 mm x 0.1 mm = 0.4 mm^3. To obtain the number of cells per mm^3 multiply the total number of cells by 2.5 (2.5 x 0.4 mm^3 = 1.0 mm^3). Record this value in Table 1.

6. WARD'S Simulated Blood has been prediluted to facilitate ease of counting. To obtain the values of each blood component per mm^3, multiply your values obtained from Step 5 by the dilution factor in Table 1 and enter these in the total count per mm^3 column.

Name:_____
Group: _____
Date: _____

ANALYSIS

Table 1
Blood Values

Component	Field 1	Field 2	Field 3	Field 4	Total # of Cells	Total # of Cells x 2.5	Dilution Factor	Total Count per mm^3
Erythrocytes								
Leukocytes:								
Neutrophils (yellow)								
Lymphocytes (blue)								
Total Leukocyte Count								
Platelets (gray/black)								

Name:_____
Group: _____
Date: _____

ASSESSMENT

1. What is the purpose of a blood smear? What information can be learned from examining a blood smear?

2. Do your blood counts differ from those given in the table of blood counts? If so, why might this be? What could be done to correct this?

3. What is a differential white blood cell count? Explain the significance of differential white blood cell counts in diagnosing diseases.

4. When preparing a blood smear, why is it important that the smear be uniform in texture and gradually decreasing in thickness?

5. Certain types of anemia result in a lower than average count for red blood cells. Do you think this method would be the easiest method for diagnosing anemia? Research other methods before deciding.

6. Create a Venn diagram comparing erythrocytes and leukocytes in human blood.

7. Place the letters from the following list next to the word to which they apply:

erythrocyte: _____	a) granulocyte
monocyte: _____	b) agranulocyte
neutrophil: _____	c) contains hemoglobin
basophil: _____	d) comes from megakaryotes
eosinophil: _____	e) lacks nuclei
lymphocyte: _____	f) capable of phagocytosis
platelet: _____	g) form of leukocyte

8. Create a poster showing the various components of blood. The poster should include descriptive information on what components are found in blood, what purpose each component serves, and relative sizes and concentrations of each component.

9. Besides the components of blood, the circulatory system carries a variety of other substances. Research other material found in the circulatory system and list some examples below.

10. Mature erythrocytes lack nuclei and mitochondria. They generate energy exclusively through a form of anaerobic metabolism. Speculate as to why you think this is.