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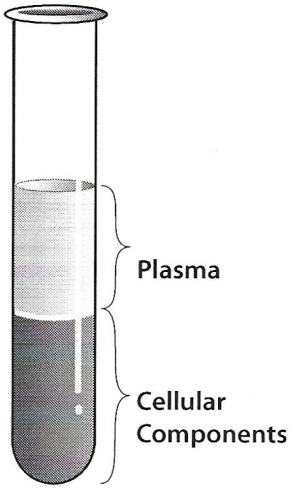
Unit 3: What's your blood type? Using Simulated Neo/BLOOD

Objectives

- ***Determine*** **the ABO and Rh blood type of unknown simulated blood samples.**
- ***Prepare*** **a simulated blood smear.**
- ***Examine*** **a prepared blood smear under the microscope to locate and identify red blood cells, white blood cells, and platelets.**
- ***Estimate*** **the number of simulated red blood cells in a given area.**

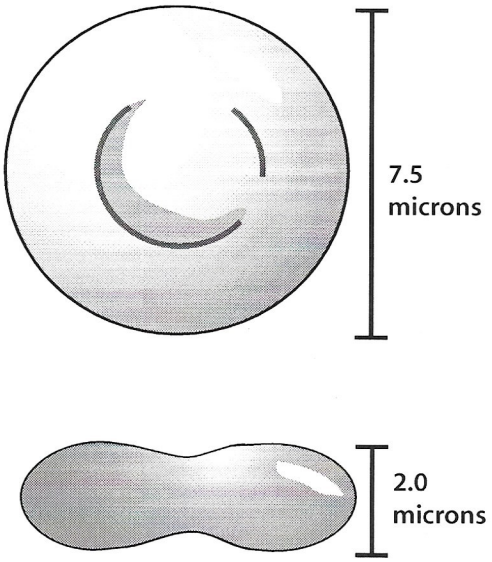
Background

Blood is a tissue comprised of four components: plasma, red and white blood cells, and platelets. Plasma is a clear straw-colored liquid portion that makes up 55% of the blood. It is composed of a mixture of water, sugar, fat, protein, and various salts. In addition, plasma contains a number of blood-clotting chemicals that help to stop bleeding.



Red and white blood cells and platelets make up the remaining 45% of the blood. Red blood cells or "erythrocytes" are tiny biconcave disks. Each red blood cell contains the oxygen-binding protein, hemoglobin. Hemoglobin contains four iron ions which bind with oxygen (O_2) and carbon dioxide (CO_2).

The shape of a red blood cell provides a greater surface area through which gases can diffuse and bind to the iron groups. The average normal red blood cell is about 7.5µm in diameter and 2µm in thickness.



Red Blood Cell

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Blood functions principally as a vehicle which transports gases, metabolic waste products and hormones throughout the body. As blood passes through the lungs, oxygen molecules attach to the hemoglobin. As blood passes through the body's tissues in capillary beds, the hemoglobin releases the oxygen. Carbon dioxide and other waste gases are, in turn, transported by the hemoglobin back to the lungs. Thereafter, the process is repeated.

Safety & Disposal

The simulated Neo/BLOOD and sera samples provided in this kit contain no biological components and are therefore safe from any potential biological hazards. However, you should always wear safety goggles, gloves and a lab apron to protect the eyes and clothing when working with any chemicals. The dye in the simulated Neo/BLOOD solution will stain your skin and clothing. Be sure that you wash your hands before leaving the laboratory.

Any simulated Neo/BLOOD waste from this lab may be disposed of by pouring it down the drain with copious amounts of water.

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ACTIVITY 1

Determining the ABO-Rh Blood Type of Simulated Blood Samples

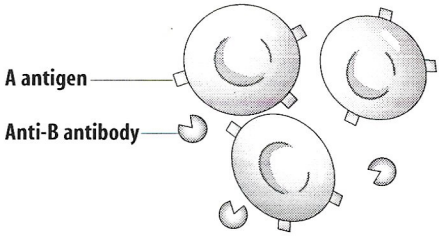
Surface proteins on red blood cells determine an individual's blood type. These surface proteins are called "antigens."

The system used to classify human blood is called the "ABO system." Dr. Karl Landsteiner, an Austrian physician, received the Nobel Prize in physiology for this discovery in 1930.

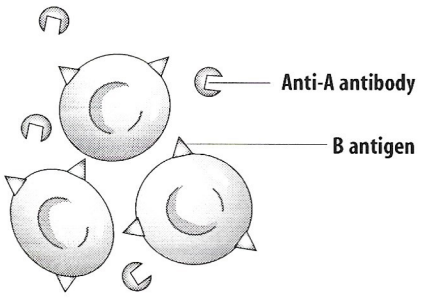
With the ABO system, the kinds of antigens present on red blood cells determines the blood type. An individual with A antigens has blood type A, one with B antigens has blood type B, one with both A and B antigens has blood type AB, and one with no antigens on the surface of his/her red blood cells has type O.

Blood plasma has circulating proteins called "antibodies". For example, individuals with A surface antigen have anti-B antibodies; those with B surface antigen have anti-A antibodies. Those with both A and B surface antigens have no antibodies. Individuals with no surface antigens have both anti-A and anti-B antibodies.

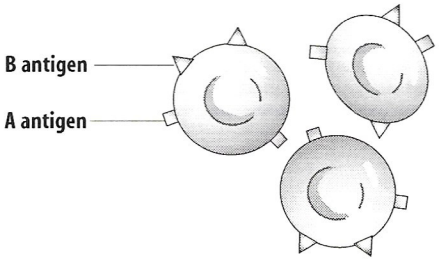
Blood typing is performed using "antiserum" - blood that contains specific antibodies. "Anti-A Serum," which contains anti-A antibodies, and "Anti-B Serum," which contains anti-B antibodies, are used in ABO blood typing.



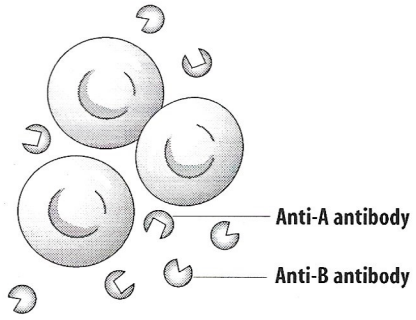
Blood Type A



Blood Type B



Blood Type AB



Blood Type O

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To perform a blood typing test, anti-A and anti-B sera are each separately mixed with a drop of sample blood and observed for "agglutination" or clumping.

Another important antigen on the surface of red blood cells is the Rh protein, named for the rhesus monkey in which it was first studied.

People who have this protein are "Rh-positive," and those who lack it are "Rh-negative."

Rh-negative individuals who have been transfused with Rh-positive blood can produce Rh antibodies. They may develop a transfusion reaction, during which agglutination may occur, if they are transfused again with Rh-positive blood. Usually Rh compatibility is tested when the ABO blood type is determined.

Data Table 2
Agglutination Reactions in the ABO System

ABO Agglutination Reaction		
Anti-A Serum	Anti-B Serum	Blood Type
Agglutination	No Agglutination	A
No agglutination	Agglutination	B
Agglutination	Agglutination	AB
No Agglutination	No Agglutination	O

Data Table 3
Rh Agglutination Reactions

Rh Agglutination Reaction	Rh Factor
Agglutination	+
No agglutination	—

Data Table 1
ABO Blood Types Summary

Blood Type	Antigen on Red Blood Cells	Antibodies in Plasma	Can Receive Blood From ...	Can Donate Blood To ...
A	A	B	O, A	A, AB
B	B	A	O, B	B, AB
AB	A and B	None	O, AB, A, B	AB
O	None	A and B	O	O, A, B, AB

Data Table 4
Frequency of ABO Blood Types and Rh Factor in the U.S.

Blood Type	Frequency Percentage	Blood Type & Rh Factor	Frequency Percentage
A	42	A +	34
		A –	8
B	10	B +	8
		B –	2
AB	4	AB +	3
		AB –	1
O	44	O +	35
		O –	9

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WARNING — This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision.

What you need

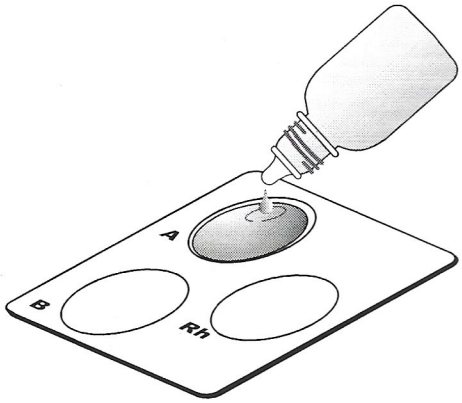
Per Student:

Anti-A Serum (simulated)	1 Blood typing tray
Anti-B Serum (simulated)	Paper towels
Anti-Rh Serum (simulated)	4 Patient Blood Samples (simulated)
	1 set Stirring sticks (blue, green and yellow)

What to do...

Step 1

Place 5 drops of Patient 1 Simulated Blood Sample in each well on your blood typing tray.



Step 2

Place 3 drops of Anti-A Simulated Serum in Well A.

Step 3

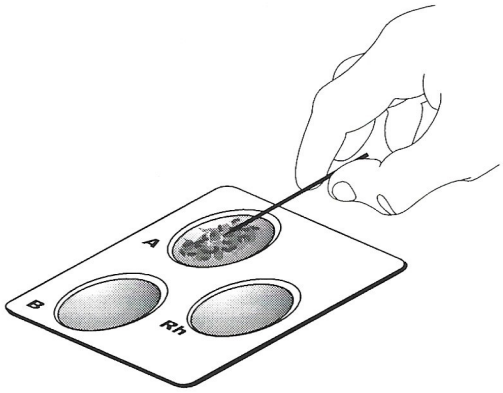
Place 3 drops of Anti-B Simulated Serum in Well B.

Step 4

Place 3 drops of Anti-Rh Simulated Serum in Well Rh.

Step 5

Use a separate stirring stick to mix the simulated blood and serum in each well for about 10 seconds.



Step 6

Carefully examine each well to determine if the simulated blood in each well has clumped or agglutinated. Record your results and observations in Data Table 1.

Step 7

Thoroughly rinse the tray and stirring sticks and repeat Steps 1-6 to type the remaining, simulated blood samples.

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Understanding Your Results:

Clumping indicates that the simulated blood sample contains antigens that reacted against the antibodies in the typing serum that you mixed it with.

Type A

If the blood in Well A is the only blood that agglutinates or clumps, then the blood sample you tested is Type A blood.

Type B

If the blood in Well B is the only blood that agglutinates or clumps, then the blood sample you tested is Type B blood.

Type AB

If the blood in both Well A and Well B agglutinates or clumps, then the blood sample you tested is type AB blood.

Type O

If the blood in both Well A and Well B does not agglutinate or clump, then the blood sample you tested is Type O blood.

Rh

If the blood in Well Rh agglutinates or clumps, then the blood sample you tested is Rh Positive blood.

Data Table 5

Simulated Blood Sample	Agglutination in Well A (+/-)	Agglutination in Well B (+/-)	Agglutination in Well Rh (+/-)	Blood Type	Observations
Patient 1					
Patient 2					
Patient 3					
Patient 4					

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Questions

1. If your blood type is B, which antigens are present on your red blood cells? What if your blood type is A, type AB, or type O?

2. Based upon your results, which ABO blood type can Patient 1 receive safely? Patient 2? Patient 3? And Patient 4?

3. Which patient is considered a universal donor?

4. How could you determine if a blood sample is compatible to transfuse from one individual to another in an emergency situation, if blood typing serum is not available?

5. How is this simulated blood typing activity, similar to actual human blood typing?

6. What would happen to a type O patient if he receives type A or B blood?

7. What are the consequences of Rh incompatibility?

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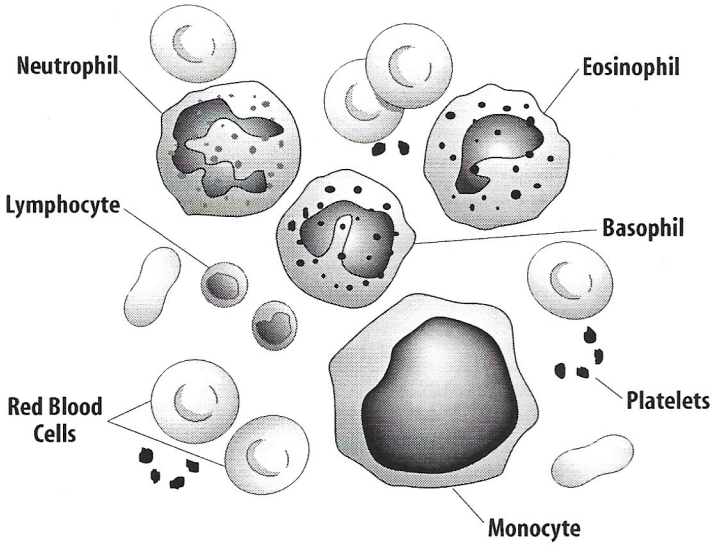
ACTIVITY 2

Taking a Closer Look at Blood

Physicians and other health care professionals regularly examine blood under the microscope to identify infections, blood cell abnormalities and to count the various types of cells.

The cells of the blood are of two classes: red blood cells (RBCs), or erythrocytes; white blood cells (WBCs), or leukocytes, which in turn are of many different types. Platelets, or thrombocytes, are also present as are cell fragments.



Red Blood Cells (RBCs)

The red blood cells are tiny, round, biconcave disks, without nuclei, that average about 7.5 microns (0.003 in) in diameter. Red blood cells, as well as most white cells and platelets, are made by the bone marrow. The main function of the red blood cells is to transport oxygen from the lungs to the tissues. A healthy 70-kg (154-lb) man has about 5 L (5.3 qt) of blood in his body, containing more than 25 trillion RBCs. The normal life span of RBCs in the circulation is only about 120 days. Worn out RBCs are removed by the spleen and liver where hemoglobin is recycled.

A number of conditions can be diagnosed based upon the red blood cell count. A high RBC level, a condition called "erythrocytosis," can be caused by smoking, living at high altitudes, or by disease. Low red blood cell levels, a condition called "anemia," can be due to a loss of blood, loss of iron, a vitamin deficiency, or other disease conditions.

Data Table 6
Normal and Abnormal Red Blood Cell Counts

RED BLOOD CELLS		
	MALE	FEMALE
Normal (at birth)	5.1 million cells per µl	4.5 million cells per µl
Normal (adult)	5.4 million cells per µl	4.8 million cells per µl
Anemia (low RBC count)	< 4.5 million cells per µl	< 4 million cells per µl
Erythrocytosis (high RBC count)	> 6.8 million cells per µl	>6 million cells per µl

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White Blood Cells (WBCs)

Leukocytes, or white blood cells, are considerably larger than red cells, have nuclei, and are much less numerous; only one or two exist for every 1,000 red blood cells, and this number increases in the presence of infection. There are three types of leukocytes, all involved in defending the body against foreign organisms: granulocytes, monocytes, and lymphocytes. There are three types of granulocytes: neutrophils (the most abundant), eosinophils, and basophils.

Platelets

Platelets (thrombocytes) are tiny bits of cytoplasm, much smaller than the red blood cells, which also lack nuclei. They are normally about 30 to 40 times more numerous than the white blood cells. They are produced as fragments of the cytoplasm of the giant cells of the bone marrow - the megakaryocytes. The platelets' primary function is to stop bleeding. When tissue is damaged, the platelets aggregate in clumps as part of the clotting process.

What you need

Blood sample (simulated)

Compound microscope

Coverslip

Microscope slide

What to do...

Step 1

Place a minute drop of simulated blood on a microscope slide.

Step 2

Place a coverslip on the simulated blood and place the slide under a compound microscope.

Step 3

Examine the simulated blood smear at 430x magnification and note the various types of simulated cells. Red blood cells will appear pink, white blood cells are stained blue, and platelets will be seen as amorphous cell fragments.

Step 4

Use the following simplified procedure to estimate the number of red simulated blood cells in 1 µl of volume of blood in a given blood sample.

Count the number of simulated red blood cells seen in your field of view. Assuming that the field of view at 430x magnification represents 0.01 µl of blood, and that the dilution factor is 1000x, then the number of simulated blood cells that would be found in 1 µl of whole blood can be calculated as follows:

$$\frac{\text{(Cells in field of view) x (Dilution factor)}}{\text{0.01 µl}} = \text{Number of Red Blood Cells in 1 µl volume of blood}$$

Example: Suppose you count 45 simulated red blood cells in the field of view, then,

$$\frac{45 \times 1000}{0.01\ \mu l} = \text{4,500,000 red blood cells per µl of blood}$$

Step 5

Select several other fields of view and repeat Step 4. Average your results to obtain a closer representative number of red blood cells per µl of blood.

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1. Based upon your results, was the red blood cell count within the accepted normal range for an adult male?

2. What does a high number of red blood cells indicate? A low level?

3. What does a high number of white blood cells indicate?

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Going Further

Red Blood Cell Count Using a Hemacytometer

The exact number of red blood cells per microliter of blood can be determined using a precision counting chamber called a "hemacytometer." Follow the instructions that accompany a hemacytometer for specific instructions on use and repeat Activity 2. Compare your blood cell counts obtained with a hemacytometer to those obtained using the simplified cell counting technique in Activity 2.

Determining the Hematocrit of a Blood Sample

Blood volume varies with body size, amount of body fat, and other factors. An average-sized adult has a blood volume of about 5 liters (1.3 gallons). If a blood-filled capillary tube is centrifuged, the red cells pack in the lower portion with the plasma floating on top. The percentage of blood volume composed of Red Blood Cells is called the "hematocrit."

Hematocrit tests are routinely performed to provide physicians with critical information related to the percent of red blood cells in a known volume of blood. This percent value can increase, for example, for individuals who are dehydrated or in shock.

The hematocrit of blood can be determined using a special microcentrifuge and hematocrit reader. Follow the instructions that accompany the microcentrifuge and reader for specific instructions on use.

Conduct library research to learn about the genetics of blood types. Also research the causes, incidence and risk factors of Rh incompatibilities, particularly during a pregnancy.

Data Table 7
Normal and Abnormal Hematocrit Values

HEMATOCRIT PERCENT			
	Normal Values	Anemia (low cell count)	Erythrocytosis (high cell count)
Male	47	<42	>54
Female	42	<32	>49

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Learn & Read More About It

Barbara J. Bain. *A Beginner's Guide to Blood Cells*. Blackwell Science, 1996.

Jackie Hardie. *Blood and Circulation*. Heinman Library, 1997.

Carol Ballard. *The Heart and Circulatory System*. Raintree/Steck Vaughn, 1997.

Arthur Selzer. *Heart: Its Function in Health and Disease*. University of California Press, 1996.

Glenn A. Langer. *Understanding Disease: How Your Heart, Lungs, Blood and Blood Vessels Function and Respond to Treatment*. Q E D Press, 1999.

Chiras, Daniel, D. *Human Biology: Health, Homeostasis, and the Environment (2nd ed.)*. West Publishing Company, 1995.

Graham, Tom M. *Biology: Life Features (2nd ed.)*. Simon & Schuster, 1998.

Knapp, Loren. *Perspectives in Human Biology*. Wadsworth Publishing, 1998.

Neat Websites

Provides information on blood banking

Http://cer.hs.washington.edu/John/blbnk.htm

Blood typing tutorial

Http://www.biology.arizona.edu/human_bio/problem_sets/blood_types/Intro.html

Information on blood and blood typing from Red Cross

http://www.redcross.org/oh/northernohio-blood/bloodtype.html

National Heart Lung, and Blood Institute

http://www.nhlbi.nih.gov/nhlbi/nhlbi.htm