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Edvo-Kit #

140

Edvo-Kit #140

Blood Typing

Experiment Objective:

The objective of this experiment is for students to understand the components of blood and relate those to the process of blood typing. Students will type blood from patients to determine which donor's blood is safe to transfuse into a patient.

See page 3 for storage instructions.

Version 140.140424

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Experiment Components

Component	Storage	Check ✓
A Control Simulated Blood Sample Type A	Room Temp.	<input type="checkbox"/>
B Control Simulated Blood Sample Type B	Room Temp.	<input type="checkbox"/>
C Control Simulated Blood Sample Type O	Room Temp.	<input type="checkbox"/>
D Unknown Simulated Blood Sample from Patient 1	Room Temp.	<input type="checkbox"/>
E Unknown Simulated Blood Sample from Patient 2	Room Temp.	<input type="checkbox"/>
F Unknown Simulated Blood Sample from Patient 3	Room Temp.	<input type="checkbox"/>
G Unknown Simulated Blood Sample from Patient 4	Room Temp.	<input type="checkbox"/>
H Anti-A Serum	Room Temp.	<input type="checkbox"/>
I Anti-B Serum	Room Temp.	<input type="checkbox"/>
J Red Dye Concentrate	Room Temp.	<input type="checkbox"/>
• Transfer pipets		<input type="checkbox"/>
• Microtiter plates		<input type="checkbox"/>
• Microcentrifuge tubes		<input type="checkbox"/>

This experiment is designed for 10 groups.

NOTE: All Control blood samples (A, B, AB & O) and Unknown Simulated Patients Blood Samples (P1, P2, P3 & P4) will be prepared by instructor just prior to use.

Experiment Requirements *(NOT included with this experiment)*

- Optional: Automatic micropipettes (5 – 50 µL) and tips

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals. No actual blood or blood products are used in this experiment. None of the experiment components are derived from human sources.

Background Information

ABOUT BLOOD AND BLOOD CELLS

Blood is a connective tissue that accounts for approximately 8% of an adult human's weight. It is composed of both fluid and cells. The fluid portion, called plasma, is approximately 55% of normal total blood volume. Of plasma, 90% is aqueous (water) and 10% is biological, consisting of different proteins and dissolved substances such as electrolytes and nutrients. Many of the proteins in plasma are involved in blood clotting and antigen detection.

The remaining 45% of blood is composed of cells. There are three main types of blood cells (Figure 1A): thrombocytes (also called platelets), leukocytes (also called white blood cells), and erythrocytes (also called red blood cells). All three of these cell types are produced in the red bone marrow from pluripotential stem cells called hemocytoblasts. "Pluri" means "many"; thus, a pluripotential stem cell is one that can differentiate into many (but not all) types of cells; in this case, hemocytoblasts can differentiate into cells that ultimately give rise to all types of blood cells. About one billion new blood cells are produced each day by a process called either hemopoiesis or hematopoiesis.

When blood is centrifuged, it separates into 3 fractions (Figure 1B). The uppermost fraction consists of plasma, a straw-colored liquid. The 2 layers beneath the plasma consist of blood cells. Immediately below the plasma is a very thin white layer called the buffy coat; this layer, which represents less than 1% of whole blood, contains leukocytes and thrombocytes. The bottom layer consists of erythrocytes and accounts for about 45% of the blood volume.

Thrombocytes

Thrombocytes (seen in Figure 2) are actually cytoplasmic fragments of large cells in the red bone marrow. Thrombocytes lack a nucleus and are thus incapable of mitosis. Adult humans have between 150,000 and 400,000 thrombocytes per μL of blood. Thrombocytes function mainly in hemostasis (i.e., stoppage of blood flow) and are commonly referred to as platelets.

Leukocytes

Leukocytes function in defense. There are between 4,800 and 10,800 leukocytes per μL of blood in an adult human. There are many different types of leukocytes. The

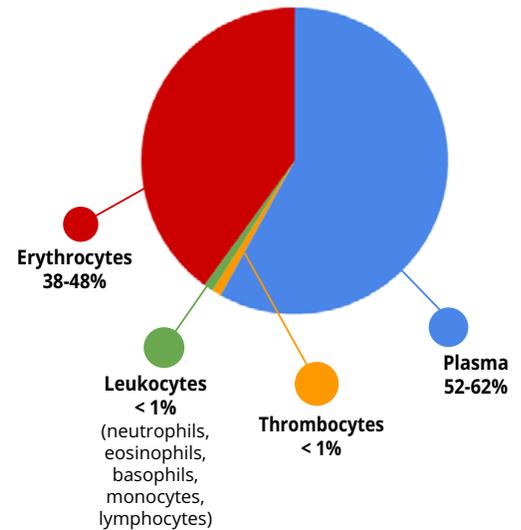


Figure 1A: Composition of Blood

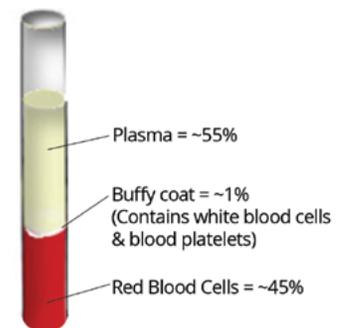


Figure 1B: Centrifuged blood

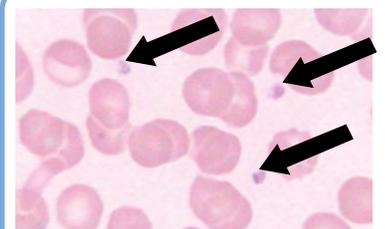


Figure 2: Thrombocytes.

main types are neutrophils, eosinophils, basophils, monocytes, and lymphocytes.

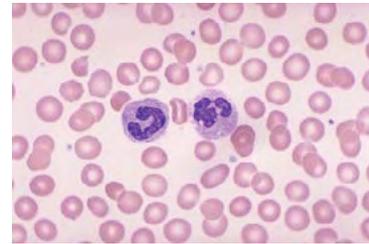
Neutrophils account for 50-70% of circulating leukocytes in humans and they measure about 10 - 12 μm in diameter. Neutrophils mainly function as phagocytes, meaning that they engulf and destroy bacteria and other material in a process known as phagocytosis. The nucleus of a neutrophil appears as a band (in less mature neutrophils) or as a segmented structure with 2-6 lobes (in more mature neutrophils). The cytoplasm of neutrophils contains numerous small, indistinct granules that contain hydrolytic enzymes and other proteins that function in defense. Both band and segmented neutrophils can be seen in (Figure 3).

Eosinophils (seen in Figure 4) account for only 2 - 4% of the circulating leukocytes in humans and are 10 - 14 μm in diameter. Eosinophils function in inflammatory processes by migrating to inflamed areas to trap substances and kill cells, thereby modulating the immune response. They are also involved in allergic disorders. Their nucleus is usually bilobed. The cytoplasm of eosinophils contains relatively large granules (as compared to neutrophils). Inside the granules are chemicals which function to destroy parasitic worms and to stop inflammatory reactions.

Basophils (see Figure 5) are the least plentiful leukocyte in humans; they comprise less than 1% of circulating leukocytes. Basophils instigate some allergic reactions. Generally smaller than neutrophils or eosinophils, basophils have a diameter of about 8-10 μm . The nucleus of the basophil, which is usually bilobed, is often not visible because of the numerous large dark blue staining (basophilic) granules in the cytoplasm. These granules contain many chemicals including heparin (an anti-coagulant) and histamine, a chemical which causes both constriction of bronchioles (air tubes within the lungs) and dilation of blood vessels.

Monocytes (seen in Figure 6) are relative large blood cells, measuring between 14 and 24 μm in diameter. About 3 - 8% of circulating leukocytes are monocytes. Monocytes function in phagocytosis to surround and kill any foreign cells and material in the body. The nucleus of a monocyte is large; either oval, indented, or folded, and appears foamy or spongy due to the arrangement of its chromatin. As the monocyte functions in phagocytosis, its cytoplasm contains many lysosomal granules. However, these granules are invisible using a light microscope; instead, the cytoplasm appears dirty gray.

Band Neutrophil



Segmented Neutrophil

Figure 3: Band and Segmented Neutrophils

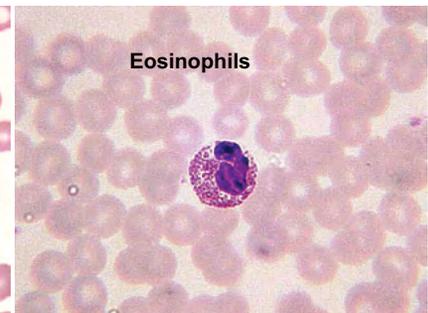
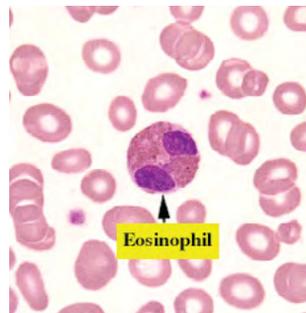


Figure 4: Eosinophils. In the image on the left, the 2 lobes of the nucleus look like 2 separate nuclei. In reality, they are 2 lobes of one nucleus that is constricted to form a thin, almost invisible central portion.

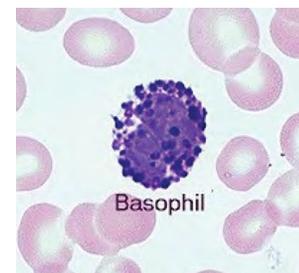


Figure 5: Basophil

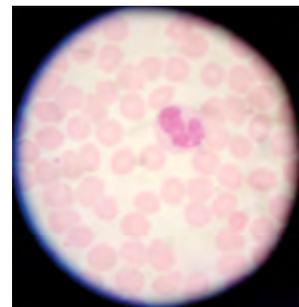


Figure 6: Monocyte

Lymphocytes (seen in Figure 7) constitute about 20-40% of circulating leukocytes. Lymphocytes function in immune responses. There are two predominant types of lymphocytes - B cells and T cells. These cells are morphologically indistinguishable from each other, but each type is unique in function and location. These cells have a diameter of 5-17 μm and have a large nucleus which is round or nearly round. Often the nucleus takes up most of the cell, making visualization of cytoplasm minimal. Irregularly shaped clumps of chromatin are visible in the nuclei of stained lymphocytes.

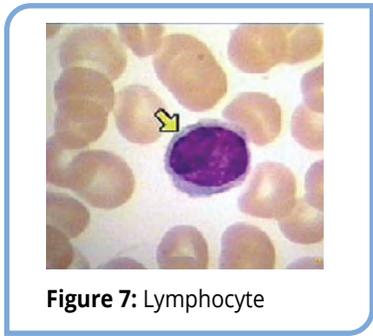


Figure 7: Lymphocyte

Erythrocytes (Red Blood Cells)

By comparing the middle and bottom layers of centrifuged blood (Figure 1B), it is easy to see that erythrocytes are the most numerous type of blood cell. Adult human females have between 4.3 and 5.2 million erythrocytes per μL of blood ($1 \mu\text{L} = 1 \text{mm}^3 = 3.4 \times 10^{-5}$ ounces = about 2.5×10^{-4} teaspoons); adult human males have between 5.1 and 5.8 million erythrocytes per μL of blood. Erythrocytes are small, measuring about $7.5 \mu\text{m}$ in diameter ($1 \mu\text{m} = 3.9 \times 10^{-5}$ inches). Erythrocytes are commonly referred to as Red Blood Cells (RBCs). Their main function is transport of oxygen and iron in the blood. RBCs are anucleate, meaning they have no nucleus. Their cytoplasm instead contains many molecules of hemoglobin (Figure 8), which binds to iron and oxygen, transporting them through the body and releasing them in tissues that are low in oxygen or iron. On the surface of RBCs are antigens which define a person's blood type. These are discussed in detail below.

ABOUT BLOOD TYPING

Blood typing depends on a precipitation reaction between an antigen and antibody. This type of reaction is called agglutination. When an antigen is attached to a red blood cell, the reaction is called an hemagglutination. Hemagglutination is a routine and cost-effective serological procedure because the agglutinate is very easily detectable.

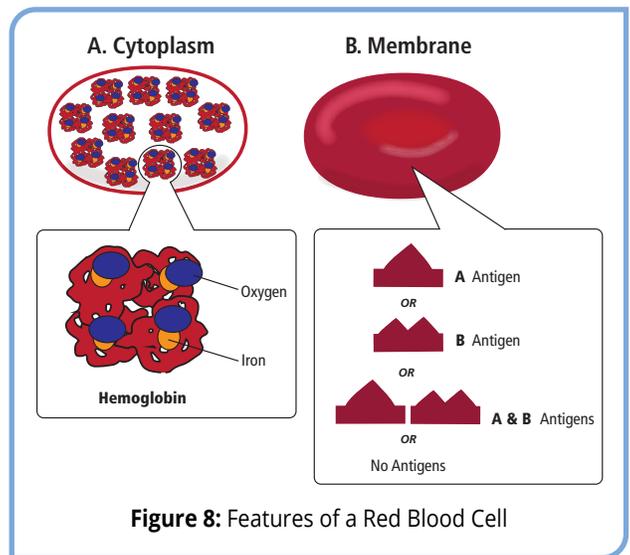


Figure 8: Features of a Red Blood Cell

Blood typing is an example of a clinical hemagglutination assay that is familiar to all of us. Blood typing has various important medical applications. The most important use of hemagglutination blood typing is to ensure safe blood transfusions, which may be needed to replace blood lost during accidents or various medical procedures.

In the hemagglutination assay, blood types of both volunteer donors and recipients are tested. After the blood typing test, the recipient is matched to a donor from whom he or she will to be able to receive blood for a safe transfusion. The antigenic determinants on the surfaces of red blood cells (RBCs) are the A, B, and O blood group antigens (Figure 8).

The two antigens provide for four possible types of blood; type A (only A antigen is on the surface of all RBCs from that person), type B (only B antigen is present); type AB (both A and B antigens are on each RBC); and O (neither A or B antigens are present). Based on the antigens on the surface of RBCs, there are four possible blood types in the ABO blood group system as listed in Table A.

Blood Type	Antigen on Red Blood Cells
A	A
B	B
AB	Both A & B
O	Neither A nor B

TABLE A: Four Different Blood Types

Blood A and B antigens are common in the human population. When exposed to the same blood group antigen, the immune system of the individual will recognize that antigen as “self” and no immune response will be mounted against it. By contrast, when exposed to different blood group antigens, the human immune system will see that antigen as foreign and produce antibodies against it. These serum antibodies can then agglutinate RBCs from individuals with a different blood type. For example, anti-A antibodies from one individual's serum will agglutinate another person's RBCs that have the A antigen on their surface. Anti-B antibodies will agglutinate RBCs that have the B antigen on their surface as demonstrated in Table B.

Type O blood is often referred to as the universal donor, and type AB blood is generally referred to as the universal recipient. However, it's not so simple. Type O blood plasma contains antibodies against both the A and B antigens. If Type O blood was transfused into a person who has Type A blood, the following ABO antigens and antibodies would be present in the recipient's blood following transfusion.

- Red blood cells with the A antigen (from recipient)
- Red blood cells with neither A nor B antigen (from donor)
- Anti-B antibodies (from recipient)
- Anti-A and anti-B antibodies (from donor)

In this case, the anti-A antibodies in the donor serum will agglutinate the recipient's red blood cells because they contain the A antigen. This can be deadly. As such, for whole blood transfusions only the same type should be transfused into a patient. Commonly though, red blood cells are isolated from the plasma by centrifugation (Figure 1b) and transfused alone. Because the red blood cells don't contain antibodies (located in plasma), Type O cells can be safely transfused into any patient.

Blood Type	Antigen on Red Blood Cells	Antibody in Serum
A	A	anti-B
B	B	anti-A
AB	Both A & B	Neither anti-A or anti-B
O	Neither A nor B	Both anti-A or anti-B

TABLE B: RBC Agglutination

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is for students to understand the components of blood and relate those to the process of blood typing. Students will type blood from patients to determine which donor's blood type is safe to transfuse into a patient.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

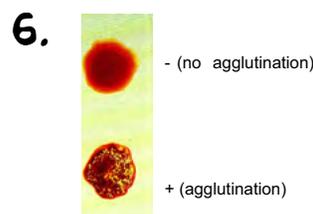
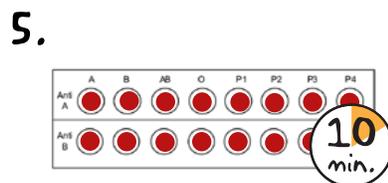
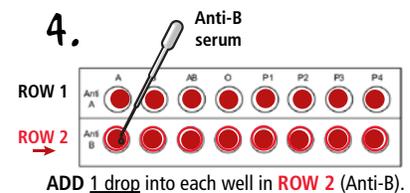
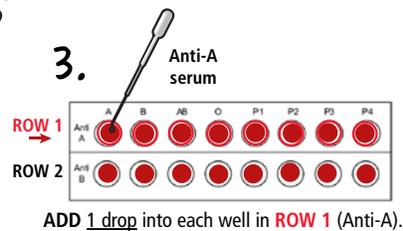
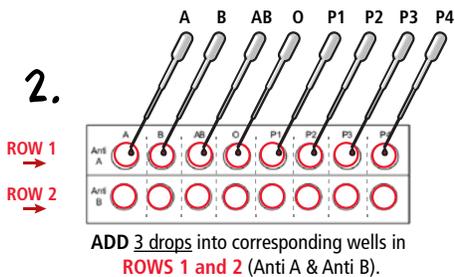
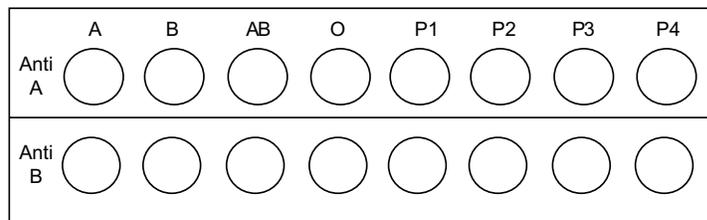
- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Hemagglutination Reaction: Transfusion Testing

1. **PLACE** one microtiter plate piece as shown below. Across the top of the plate, **LABEL** the 8-wells A, B, AB, O, P1, P2, P3, and P4 respectively, using a laboratory marking pen. **LABEL** the 2 rows Anti-A and Anti-B respectively. The plate should look like the one pictured below.



2. Using a different pipet or pipet tip for each sample, **PLATE** 3 drops of each control blood type and patient sample into each of the two corresponding wells. For example, Control A blood type goes into the two wells under the letter A. Each well requires 3 drops or 50 μL . **IMPORTANT: Avoid cross-contamination by using a new disposable pipet or pipette tip for each sample.**
3. Use a new pipet to **ADD** one drop or 20 μL of Anti-A serum into each of the wells in row #1.
4. Use a new pipet to **ADD** one drop or 20 μL of Anti-B serum into each of the wells in row #2.
5. Let the plate **INCUBATE** undisturbed on the lab bench for 5-10 minutes.
6. **OBSERVE** the wells for the presence or absence of agglutination. Agglutination has occurred if the mixture appears to be granular rather than smooth. **RECORD** your results in the diagram in the Results section.

Analysis

1. Record your results in the diagram below:

	A	B	AB	O	P1	P2	P3	P4
Anti A	<input type="checkbox"/>							
Anti B	<input type="checkbox"/>							

2. What are the ABO blood types of the four patients?
3. Which donor's whole blood could be safely transfused into patient #1?
What about a red blood cell transfusion?

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What is the difference between agglutination and hemagglutination?
2. What is the composition of blood?
3. If a patient has type B blood, what blood type should be given to them for whole blood transfusion? What about red blood cell transfusion? Why?
4. What are the basic blood types?

Instructor's Guide

NOTES TO THE INSTRUCTOR

Blood typing is an important clinical assay that health care workers use routinely to properly care for their patients. Students should be made aware of the safety concerns when working with human blood products even though all the materials in this EDVOTEK kit are chemicals used to simulate blood.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK website. In addition, Technical Service is available from 8:00 am to 5:30 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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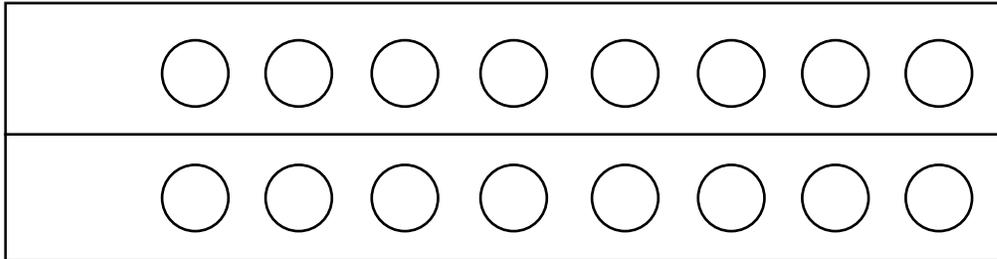
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Pre-Lab Preparations

Preparing the Microtiter Plate

Each group will require one microtiter plate piece (2 rows of 8 wells).



Preparation of Control and Patient Blood Samples

(Prepare no more than 24 hours before starting the experiment.)

1. Retrieve the Control and Patient Simulated Blood Samples (Components A-G). Add 4 drops or 50 μL of Red Dye Concentrate (Component F) to each sample.
2. To prepare Control blood sample AB, combine 700 μL of Control blood sample A and 700 μL of Control blood sample B (prepared in step 1) in a labeled microcentrifuge tube. Cap and mix well.
3. Label microcentrifuge tubes:
 - 10 tubes "A"
 - 10 tubes "B"
 - 10 tubes "AB"
 - 10 tubes "O"
 - 10 tubes "P1"
 - 10 tubes "P2"
 - 10 tubes "P3"
 - 10 tubes "P4"
4. Aliquot 100 μL of each Control and Patient blood samples (prepared in steps 1 and 2) to the appropriately labeled tubes. Use a new pipet or pipet tip for each sample.
5. Label 10 tubes "anti-A". Aliquot 180 μL of Anti-A serum (Component H) to each.
6. Label 10 tubes "anti-B". Aliquot 180 μL of Anti-B serum (Component G) to each.
7. Students will also require automatic micropipets and tips or 10 transfer pipets for dispensing the samples.

**Please refer to the kit
insert for the Answers to
Study Questions**

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insert for the Answers to
Study Questions**