EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit

140

Blood Typing

Store entire experiment at room temperature.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to learn the concept of blood typing. A second objective is for students to differentiate between various types of cells found in blood and briefly provide an overview of their functions.

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Table of Contents

Material Safety Data Sheets can be found on our website:

www.edvotek.com

This experiment is designed for 10 student groups.

No actual blood or blood products are used in this experiment.

Experiment Components

Store entire experiment at room temperature.

- Control ABO simulated blood samples (A, B, & O)**
- Unknown simulated blood samples
  from four patients (P1, P2, P3 & P4)
- Anti-A and Anti-B serum
- Red dye concentrate (for coloring)

- Transfer pipets
- Microtiter plates
- Microcentrifuge tubes

** ***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.***

Requirements

- Optional: Automatic micropipet (5 - 50 µl) and tips

Background Information

Precipitation reactions between soluble antigens and antibodies can be visible reactions if both components are in equivalence. Under this condition neither the antigen nor the antibody is in excess and antigen-antibody complexes form large networks that precipitate out of solution as shown in Figure 1, below.

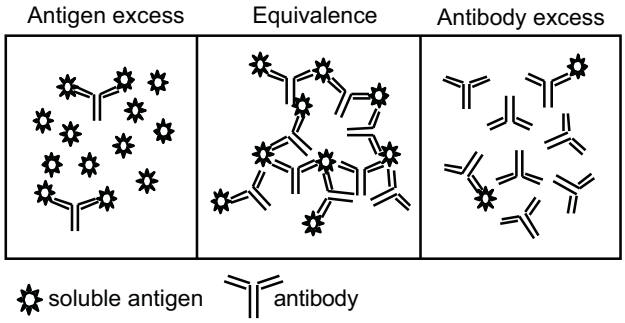


Figure 1: Antigen and antibody reactions

When an antigen is attached to a red blood cell, the reaction is called an agglutination and the lattice of antigen and antibody that is visible at equivalence is called an agglutinate. Agglutination is a routine and cost-effective serological procedure because the agglutinate is very easily detectable.

Blood typing is an example of a clinical agglutination assay that is familiar to all of us. Since the specific blood antigens are on the surface of red blood cells (RBCs) they are termed hemagglutination reactions. 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 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 proteins, which are for convenience called A, B, and O antigens.

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, below.

Blood Type	Antigen on Red Blood Cells
A	A
B	B
AB	both A and B
O	neither A nor B

Table A: 4 different blood types

Background Information

Blood A and B antigens are common in the human population, as well as in nature, including bacteria to which we are exposed. When exposed to bacteria with 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 bacteria with 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, below.

Blood Type	Antigen on Red Blood Cells	Antibody in Serum
A	A	anti-B
B	B	anti-A
AB	both A and B	neither anti-A nor anti-B
O	neither A nor B	both anti-A and anti-B

Table B: RBC Agglutination

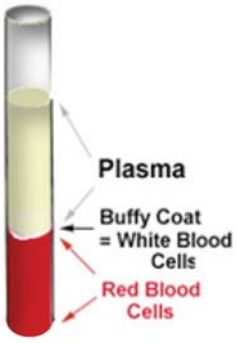
Type O blood is often referred to as the universal donor, and type AB blood is generally referred to as the universal recipient. Neither is correct. There is no universal donor nor a universal recipient in the case of whole blood transfusions. Type O blood is commonly said to be the universal donor because type O RBCs do not have either A or the B antigen on their surface. Thus Type O red blood cells were incorrectly assumed to be safe for transfusing individuals with Type A, B or AB blood. This assumption is incorrect and can have serious medical consequences where the anti-A antibodies from the donor would react with the recipient's red blood cells. Therefore, Type O blood is a universal donor only if red blood cells (and not serum) are being transfused.

If Type O blood was transfused into a person who has Type A blood, the following ABO antigens and antibodies would be present in various 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)

Therefore, in reality, only blood of the same type should be transfused into a patient. In fact, since there are subtypes of some of the blood groups and since there are other blood groups besides ABO which may cause transfusion problems, even this conservative approach is an oversimplification and may result in complications for the patient.

Background Information



Centrifuged blood

Plasma = ~ 55%

Buffy coat = < 1%
contains white blood cells
& blood platelets

Red Blood Cells = ~ 45%

ABOUT BLOOD AND BLOOD CELLS

Blood is a connective tissue that accounts for about 8% of an adult human's weight. It is composed of both fluid and cells. The fluid portion, called plasma, is 90% aqueous (water) and is approximately 55% of normal total blood volume. Biological components in plasma amount to 10% and include different proteins and dissolved substances such as electrolytes and nutrients. Some of the proteins in plasma are involved in blood clotting; when these proteins are removed from the plasma, the resultant liquid is called serum. In effect, plasma is the fluid portion of whole blood, and serum is the fluid portion of clotted blood.

The remaining 45% of blood is composed of blood cells. There are three main types of blood cells: erythrocytes (also called red blood cells), thrombocytes (also called platelets), and leukocytes (also called white 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 but not to other cells in the body.) 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 (see Figure 2). 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.

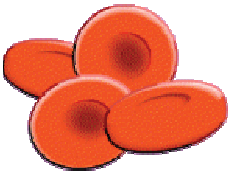


Figure 3: Erythrocytes

By comparing the middle and bottom layers of centrifuged blood, it is easy to see that erythrocytes are the most numerous type of blood cell. Erythrocytes (seen in Figure 3) function in the transport of oxygen and, to a lesser degree, carbon dioxide. Adult human females have between 4.3 and 5.2 million erythrocytes per µl of blood (1 µl = 1 mm^3 = 3.4 x 10^{-5} ounces = about 2.5 x 10^{-4} teaspoons); adult human males have between 5.1 and 5.8 million erythrocytes per µl of blood. Mature erythrocytes do not have a nucleus; as such, they are incapable of cell division. Erythrocytes are small, measuring about 7.5 µm in diameter (1 µm = 3.9 x 10^{-5} inches). The center of an erythrocyte is thinner than its periphery; thus, the center appears lighter than the periphery.

Thrombocytes (seen in Figure 4) are actually cytoplasmic fragments of large cells in the red bone marrow. Like erythrocytes, 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).

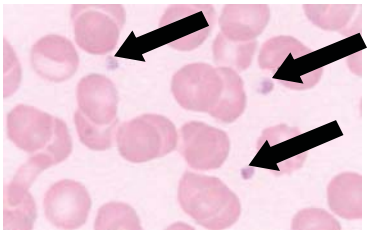


Figure 4: Thrombocytes.

Background Information

Leukocytes function in defense. There are between 4,800 and 10,800 leukocytes per µl of blood in an adult human. Neutrophils, which function mainly in phagocytosis, account for 50-70% of circulating leukocytes in humans; they measure about 10 - 12 µm in diameter. 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). Because of the variation in the shape of the nucleus, neutrophils are sometimes called polymorphonuclear leukocytes ("poly" means "many"; "morpho" means "shape") or simply polys or polymorphs. Because neutrophils are phagocytes, they are sometimes called microphages (meaning "small eaters"). The cytoplasm of neutrophils contains numerous small, indistinct granules that contain hydrolytic enzymes or other proteins that can function in defense. Because the granules of neutrophils stain with both an acid and a basic stain, they are considered neutral - thus, the name neutrophil. The granules of neutrophils appear light pink on a stained slide. Both band and segmented neutrophils can be seen in (Figure 5).

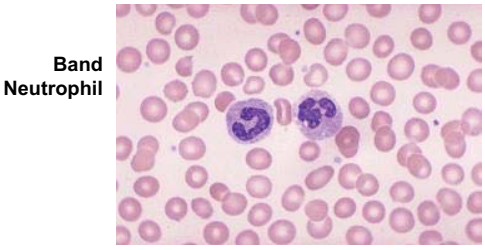


Figure 5: Band and Segmented Netrophils

Eosinophils (seen in Figure 6) account for only 2 - 4% of the circulating leukocytes in humans and are 10 - 14 µm in diameter. Their nucleus is usually bilobed. The cytoplasm of eosinophils contains relatively large granules (as compared to neutrophils); as the name of the cell implies, the granules stain with eosin. Thus, the granules are acidophilic (meaning "acid loving") and appear bright reddish-orange on a stained slide. Inside the granules are chemicals which function to destroy parasitic worms and to stop inflammatory reactions.

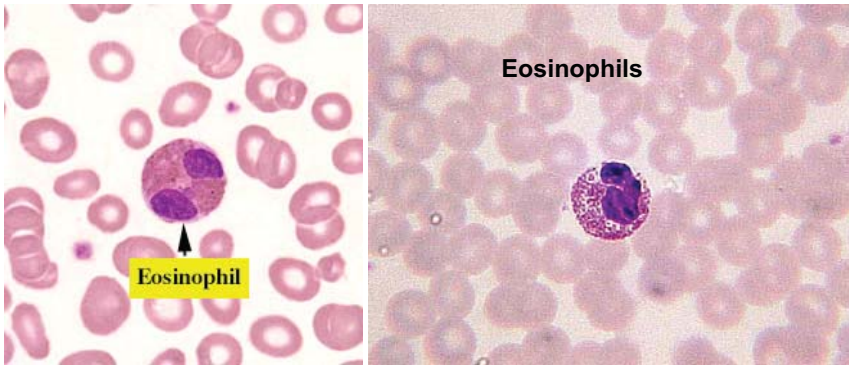


Figure 6: Eosinophils. In the image on the left, the 2 lobes of the nucleus look like 2 separate nuclei. In reality, they are 2 lobes of one nucleus that is constricted to form a thin, almost invisible central portion.

Background Information

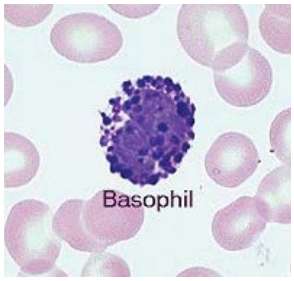


Figure 7: Basophil

Basophils (see Figure 7) are the least plentiful leukocyte in humans; they comprise less than 1% of circulating leukocytes. 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). Basophils instigate some allergic reactions.

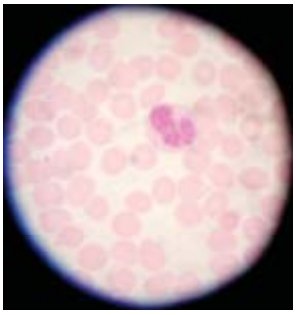


Figure 8: Monocyte

Monocytes (seen in Figure 8) are relative large blood cells, measuring between 14 and 24 µm in diameter. About 3 - 8% of circulating leukocytes are monocytes. 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. Monocytes that leave the blood and enter the tissues are called macrophages (meaning "big eaters"). Monocytes and macrophages are the major phagocytes in the body.

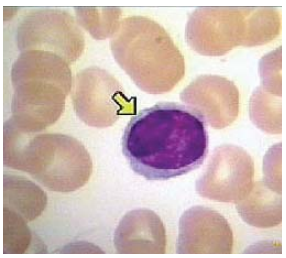


Figure 9: Lymphocyte

Lymphocytes (seen in Figure 9) constitute about 20-40% of circulating leukocytes. 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. 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. In terms of function, B cells produce antibodies while T cells function in cell-mediated immunity and in controlling and regulating immune responses. Because of their differences in function, B and T cells express different cell surface receptors and thus can be separated from each other in the laboratory even though they appear identical. In terms of location, the majority of B cells home to specialized areas of the body called lymphoid tissues (such as red bone marrow, lymph nodes, the spleen, tonsils and the appendix); a lesser number of B cells are found in the blood. By contrast, most T cells circulate in the blood, while a minority of T cells home to lymphoid tissues.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to learn the concept of blood typing. A second objective is for students to differentiate between various types of cells found in blood and briefly provide an overview of their functions.

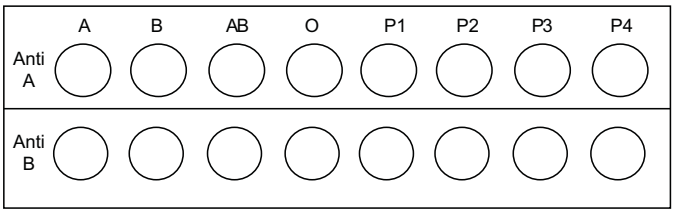
LABORATORY SAFETY

No human materials are used in this experiment. Gloves and safety goggles should be worn as good laboratory practice.

Student Experimental Procedures

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.



IMPORTANT! Avoid cross-contamination by using a new pipet or pipet tip for each blood sample.

PUT ON YOUR GLOVES NOW.

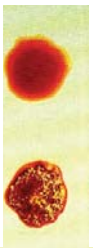
2. Using a different pipet or pipet tip for each sample, plate 3 drops of each control blood type and patient sample into each of the two corresponding wells. For example, control A blood type goes into the two wells under the letter A. Each well requires 3 drops or 50 µl.

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 sit 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.



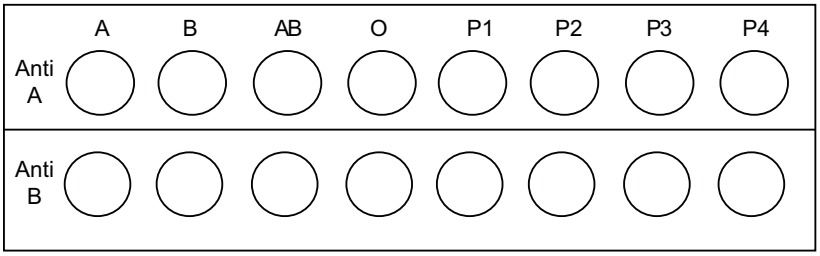
- (no agglutination)

+ (agglutination)

Experiment Procedure

Student Experimental Results

1. Record your results in the diagram below.



2. What are the ABO blood types of the four patients?

3. Which donor's blood could be safely transfused into patient #1?

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. What is the difference between blood plasma and blood serum ?

4. What are the basic blood types?

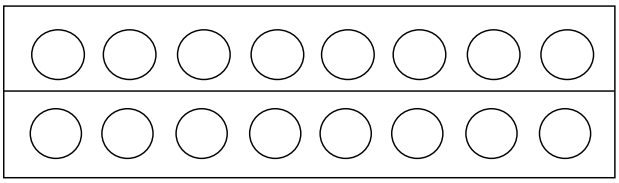
Instructor's Guide

GENERAL INFORMATION

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 of the materials in this Edvotek kit are chemicals used to simulate blood.

Pre-Lab Preparations

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



B. Preparation of Control and Patient Blood Samples
(Prepare no more than 24 hours before starting the experiment.)

1.a. To prepare the Control blood samples A, B & O and Unknown Simulated Patients Blood Samples P1, P2, P3 & P4, add 4 drops or 50 µl of Red dye concentrate to the appropriate blood sample provided in the kit. Cap tubes and mix well.

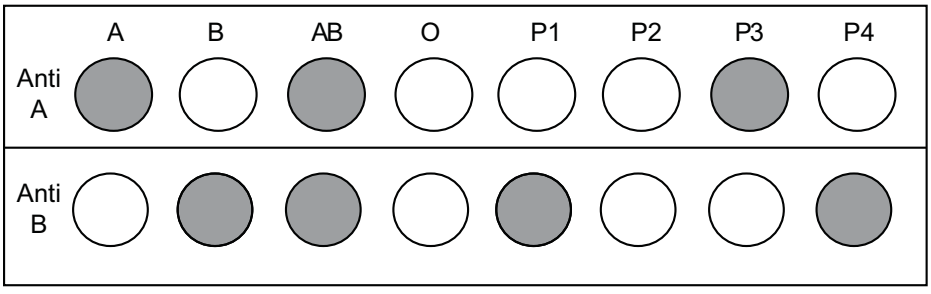
1.b. To prepare Control blood sample AB, combine 700 µl of Control blood sample A and 700 µl of Control blood sample B (prepared in step 1.a.) in a labeled microcentrifuge tube. Cap and mix well.

2. Label microcentrifuge tubes "A", "B", "AB", & "O", "P1", "P2", "P3". "P4". Aliquot 100 µl of each Control and Patient blood samples (prepared in steps 1.a. & 1.b.) to the appropriately labeled tubes. Use a new pipet or pipet tip for each sample.

3. Label tubes "anti-A" and "anti-B" and aliquot 180 µl of each to the respective tubes.

4. Students will also require automatic micropipets and tips or 10 transfer pipets for dispensing the samples.

Expected Results



Instructor's Guide

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