

NAME \_\_\_\_\_

DATE \_\_\_\_\_

## ABO-Rh Blood Typing with Synthetic Blood

In this lab, you will determine the blood type of four different synthetic blood samples using antisera to the A, B, and Rh (D) antigens that exist on human red blood cells. The procedure for the blood test is the same that would be used for a real blood test, but, for convenience and safety, the blood and antisera are synthetic and contain no biological materials.

### Background

By volume, blood tissue is approximately 55% plasma and 45% cells. Plasma is a straw-colored liquid consisting of 90% water and a variety of salts and proteins important for maintaining osmotic balance, buffering against pH changes, maintaining blood viscosity, transporting certain materials, and for blood clotting when a blood vessel is injured.

There are three major types of **blood cells**—red blood cells (erythrocytes), white blood cells (leukocytes), and platelets.

**Red blood cells**, the most numerous cells in the blood, carry oxygen from the lungs to all parts of the body. A red blood cell is a biconcave disk with a thin center. This shape provides a large surface area for diffusion of oxygen. Red blood cells contain the protein hemoglobin. Iron is incorporated into the hemoglobin molecule. When blood travels through the lungs, the oxygen in the lungs combines with the iron in hemoglobin. When the blood moves through the body's capillary system, the oxygen carried in the red blood cells is released from the iron in hemoglobin to the other cells of the body. Red blood cells' flexibility allows them to pass through even the smallest blood vessels.

**White blood cells** make up only about 1% of the blood volume. They are an important part of the **immune system**. Their primary function is to provide defense against invaders in the body, which may include bacteria, parasites, fungi, and viruses. White blood cells may attack a foreign body directly, they may produce antibodies that identify, attach to, and neutralize a foreign body, or they may trigger other cells to act in destroying the foreign body.

**Platelets** perform a vital function in the process of coagulation, or blood clotting, which occurs when a blood vessel is injured.

### Blood Types

Although the basic composition and function of blood in each of us is the same, there are different human blood types. The cell membrane of red blood cells, like that of other cells, has molecules that project from its surface. Some of the molecules function as identification badges, allowing the immune system to recognize the cell as a normal component of an individual's body. If blood from a person whose red cells have different surface molecules is injected into someone, those molecules are recognized as foreign to the body, or **antigenic**. The immune system attacks the antigens and attempts to destroy them and the cells that carry them. This is why transfusion with an incompatible blood type is harmful. The recipient's body recognizes the antigens on the transfused red blood cells as foreign and attacks and destroys the cells. For that reason, donated blood is thoroughly tested for A, B, O, and Rh antigens and is transfused only into compatible recipients.

## The ABO Blood Groups

The ABO blood groups (types) result from the presence or absence of two antigens, A and B, on the surface of the red blood cells. If antigens are present very early in life, the immune system recognizes those antigens as "self" and will not generate an immune response to them. As a result, the body does not generate antibodies to any A and B antigens present on its own blood cells. However, the immune system does produce antibodies to any A and B blood antigens not present on the organism's own cells. Type A blood has the A antigen on its red blood cells and anti-B antibodies in the plasma. Type B blood has the B antigen on its red blood cells and A antibodies in the plasma. Type AB blood has both A and B antigens on the red blood cells and no antibodies in the plasma. Finally, Type O blood has neither A nor B antigens on the red blood cells and both A and B antibodies in the plasma. These antibodies are present even if the person has not had any foreign blood introduced into their body. It is hypothesized that the antibodies are present because of similarity between the A and B blood antigens and other antigens present in the environment. If two antigens are similar enough, the antibodies generated to one antigen will also recognize the other.

The relationships of the ABO blood types to the presence of antigens and antibodies in the blood are summarized in the table that follows.

## The Rh Blood Groups

Another important antigen found on the surface of blood cells is the Rh factor. The Rh antigen is actually a whole group of closely related antigens. Blood containing an Rh antigen is said to be Rh positive (Rh+); blood lacking the antigen is said to be Rh negative (Rh-). Unlike the case for the ABO antigens, the production of Rh antibody requires prior exposure to the antigen, such as would occur in an Rh- pregnant woman carrying a fetus that was Rh+.

Blood Group	Red Cell Antigen Present on Cells	Antibody that Recognizes Cells
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	anti-A and anti-B

## Pre-laboratory Questions

- Shown in the table below are the antigens present on the blood cells of four different people. Fill in the corresponding ABO blood type for each person and the antibodies expected to be found in each person's blood.

Blood Type	Red Cell Antigen	Antibody in Blood Plasma
	A	
	B	
	AB	
	Neither	

2. For each blood type in the table below, fill in the expected agglutination result from mixing the blood with each of the antibodies.

Blood Type	Anti-A	Anti-B	Anti-Rh
A+			
A-			
B+			
B-			
AB+			
AB-			
O+			
O-			

### Materials

vial synthetic anti-D (anti Rh) serum  
 vial synthetic anti-A serum  
 vial synthetic anti-B serum  
 blood typing slide  
 4 blue mixing sticks  
 4 yellow mixing sticks  
 4 white mixing sticks

vial of Sample 1 synthetic blood  
 vial of Sample 2 synthetic blood  
 vial of Sample 3 synthetic blood  
 vial of Sample 4 synthetic blood  
 paper towels  
 access to tap water and a sink

### Procedure

- Using the dropper vial, place a drop of the Sample 1 synthetic blood sample in each well of the blood typing slide. Close the cap on the dropper vial. To prevent cross contamination, always close the cap on one vial before opening the next vial.
- Add a drop of synthetic anti-A serum (blue) to well A. Close the cap.
- Add a drop of synthetic anti-B serum (yellow) to well B. Close the cap.
- Add a drop of synthetic anti-Rh serum (clear) to well Rh. Close the cap.
- Using a different-colored mixing stick for each well (blue for anti-A, yellow for anti-B, white for anti-Rh), gently stir the synthetic blood and antiserum drops for 30 seconds. Remember to use a new mixing stick for each sample to avoid contamination of your samples.
- Carefully examine the resulting thin films of liquid mixture in the well. If a film is uniform in appearance, there is no agglutination. If the sample appears granular, agglutination has occurred.
- Fill in the column for Sample 1 in the following data table, answering yes or no as to whether agglutination occurred with each antiserum.
- Thoroughly rinse the blood typing slide and then repeat steps 1 through 7 for synthetic blood Samples 2, 3, and 4.

**Data Table**

	Sample 1	Sample 2	Sample 3	Sample 4
Anti-A				
Anti-B				
Rh				
Blood Types				

**Questions**

- At 1:00 a.m., someone breaks a window in the back of a store and robs the safe. On the way out, the thief is cut on a piece of broken glass. A forensic team collects and tests a sample of blood left behind by the thief. It is O-. The police bring in a suspect with a cut forearm who was arrested just three blocks from the store. The person resembles someone seen leaving the store at the time of the robbery. A sample of the suspect's blood is taken and tested for blood type. Once the suspect's blood is mixed with anti-A serum, it is immediately clear that the suspect is not the person who was cut on the broken glass in the store. How did the test indicate that fact?
  
- Suppose the same suspect's blood does not agglutinate when tested with anti-A or anti-B, but does agglutinate when tested with anti-Rh. Would this connect the suspect with the crime scene? Explain your answer.

3. Tom and Jane participate in a Red Cross blood drive. Both are first-time donors. As part of the screening process, their blood is typed. Tom is A+. Jane is AB+.
  - a. What ABO antibody is found in Tom's blood?
  
  
  
  
  
  
  
  
  
  
  - b. What ABO antigens are found in Jane's blood?
  
4. The same Tom and Jane's blood donations are sent to a processing center where the blood cells are separated from the plasma in each of the two samples. The separated cells and plasmas are then sent to a hospital. A blood researcher wishes to use Tom's blood in an attempt to extract and identify the A antigen. Should she attempt the extraction process on his blood cells or on his plasma?

## Blood Typing Lab

### Scenario:

A murder has taken place. There are two suspects in custody. Suspect #1 was the victim's neighbor and had reported the victim to the police department multiple times for failure to clear his sidewalk after snowstorms. Suspect #2 is the victim's coworker who missed a promotion due to the victim's poor review. At the crime scene a bloodstain was found, not believed to have been the victim's. Your job is to compare the blood samples of the two suspects to that of the crime scene and the victim. Determine which of the two suspects left behind the bloodstain.

### Materials Needed:

4 blood typing trays

Suspect #1 sample

Suspect #2 sample

Victim sample

Crime scene sample

Anti-A serum

Anti-B serum

Anti-Rh serum

Toothpicks

Blood Type/Antisera Reactions

	O-	O+	A-	A+	B-	B+	AB-	AB+
Anti-A	-	-	+	+	-	-	+	+
Anti-B	-	-	-	-	+	+	+	+
Anti-Rh	-	+	-	+	-	+	-	+

### Procedure:

Label your blood typing trays

Place 2 drops of each type of blood in well A, B and Rh of the sample tray.

Place 2 drops of anti-A, anti-B and anti-Rh into the respective wells. Record the results. Clean your sample trays.

	Victim	Suspect 1	Suspect 2	Crime scene
Anti-A				
Anti-B				
Anti-Rh				

Write your conclusion using the following: Could the sample have been from either suspect? Could the sample be from the victim?