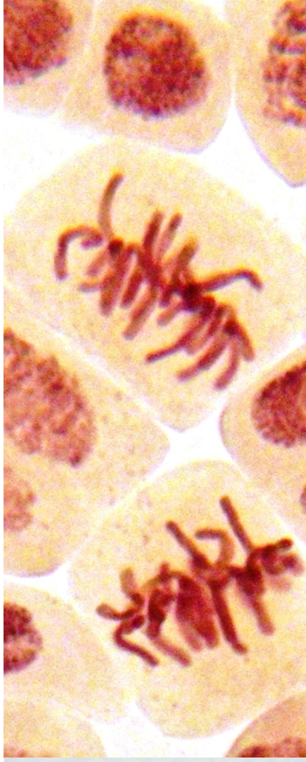
Innovating Science®

by Aldon Corporation

"cutting edge science for the classroom"



Instruction Manual

ABO/Rh Blood Typing Activity

IS3101

221 Rochester Street
Avon, NY 14414
Phone: (585) 226-6177
Fax: (585) 226-6919
www.aldon-chem.com

ALDON®

Next Generation Science Standards

MS-PS1-2. Analyze and interpret data on the properties of substances before and after the substances interact to determine if a chemical reaction has occurred.
[Clarification Statement: Examples of reactions could include burning sugar or steel wool, fat reacting with sodium hydroxide, and mixing zinc with hydrogen chloride.]

PS1.A: Structure and Properties of Matter

- Each pure substance has characteristic physical and chemical properties (for any bulk quantity under given conditions) that can be used to identify it.

PS1.B: Chemical Reactions

- Substances react chemically in characteristic ways. In a chemical process, the atoms that make up the original substances are regrouped into different molecules, and these new substances have different properties from those of the reactants.

Aligned to the Next Generation Science Standards (NGSS)*

ABO/Rh Blood Typing Activity

IS 3101

INTRODUCTION

Blood typing is a method of classifying blood based on the presence or absence of specific proteins on the surface of red blood cells, or erythrocytes. Blood type, an inherited characteristic, is valuable to know in that it affects medical procedures, such as surgery and transfusions, paternity testing, as well as serving as evidence in criminal investigations.

ABO and Rh Blood Typing

There are currently 26 different blood typing systems based on proteins on the surface of erythrocytes that lead to an individual's blood type. However, the most important and commonly used system for the classification of blood is the ABO/Rh system.

In the late 1800s, an Austrian physician by the name of Karl Landsteiner noted that human blood, when transfused into other animals, would clump up, or agglutinate, in the blood-carrying vessels of the recipient animal, resulting in death due to lack of blood circulation. Simply knowing this was not enough for Landsteiner. He needed to know why it occurred, as Landsteiner realized that if this reaction happened in the case of a human blood transfusion, the results would be disastrous.

Continuing his research, in the early 1900s Landsteiner discovered two distinct proteins that may or may not be on the surface of an individual's blood cells. He termed these proteins A and B. Landsteiner recognized four distinct blood types due to the presence or absence of these molecules. Blood cells containing only the A molecule, Landsteiner called blood type A; blood cells with only the B molecule, he called blood type B; blood cells with both molecules, he named blood type AB, and, in some cases, neither molecule was present, and Landsteiner termed this blood type O.

These proteins turned out to be a form of antigen, a substance that can stimulate an immune response in an individual. This is very important because more research showed that a person has specific antibodies (substances that attack antigens in an immune response) in the liquid portion (plasma) of their blood. The antibodies in the plasma are determined by the antigens present on the erythrocytes. An individual with blood type A has anti-B antibodies; someone with blood type B has anti-A antibodies; blood type AB produces neither antibody in the plasma, and a person with blood type O has both anti-A and anti-B antibodies in their plasma.

As mentioned earlier, this is of vital importance because antibodies are manufactured by the immune system to attack foreign substances within the body. If, for example, a person with blood type A (and therefore anti-B antibodies in their plasma) is given a blood transfusion of type B blood, the anti-B antibodies will recognize these blood cells as foreign and attack them, resulting in agglutination and most probably death. If this same individual is given a transfusion of type A blood, the anti-B antibodies will not recognize the blood cells and the transfused blood will function properly in the recipient's circulatory system.

In the case of blood type O, neither A nor B antigens are present on the erythrocytes. This means that blood cells of type O can be safely transfused into anyone, as there is nothing for antibodies to recognize. A person with blood type O is called a universal donor. Conversely, in the case of blood type AB, neither anti-A nor anti-B antibodies are present in the blood plasma. Therefore, an individual with blood type AB can receive a transfusion of any blood type, with no risk of antibodies being present to attack the transfused cells. A person of blood type AB is known as a universal recipient.

Table 1: ABO Blood Types

Blood type	Can donate to	Can receive
A	A, AB	A, O
B	B, AB	B, O
AB	AB	AB, A, B, O
O	AB, A, B, O	O

Furthering his research, Landsteiner, working with another scientist named Alexander Wiener, discovered that when blood from a Rhesus monkey was transfused into a rabbit, the rabbit would produce antibodies that would also attack certain human blood cells. Again it was discovered that this reaction was triggered by the presence of antigens on the blood cells and since these antigens were discovered on the blood cells of the Rhesus monkey, Landsteiner and Wiener termed this the Rh factor. An individual with Rh antigens on their erythrocytes is termed Rh+ and a person lacking Rh antigens is called Rh-. Again, this is very important because it dictates whether or not an individual will have an immune response to certain types of blood.

Table 2: ABO/Rh Blood Types

Blood type	Can donate to	Can receive
A+	A+, AB+	A+, A-, O+, O-
A-	A+, A-, AB+, AB-	A-, O-
B+	B+, AB+	B+, B-, O+, O-
B-	B+, B-, AB+, AB-	B-, O-
AB+	AB+	AB+, AB-, A+, A-, B+, B-, O+, O-
AB-	AB+, AB-	A-, B-, AB-, O-
O+	A+, B+, AB+, O+	O+, O-
O-	A+, A-, B+, B-, AB+, AB-, O+, O-	O-

As mentioned above, 26 different antigen systems have been recognized on the surface of red blood cells, however, the ABO/Rh system is the most widely used and important system in the case of blood transfusions. So important, in fact, that Landsteiner's discovery was further validated when he was awarded the Nobel Prize for medicine in 1930.

Determining ABO/Rh Blood Type

Blood type can easily be determined in the lab. To do so, a sample of the blood is placed in a special blood typing tray that contains wells labeled “A,” “B,” and “Rh.” The blood then has anti-serum added to it. The anti-serum has antibodies in it that react against specific antigens on the surface of red blood cells and cause the sample to agglutinate in the well of the blood typing tray. Like the three labeled wells on the blood typing tray, there are three types of anti-sera: anti-A, anti-B, and anti-Rh. Each is added to the corresponding well on the tray.

Each of the three wells is examined for agglutination and the blood type may be determined depending on which of the wells, if any, show the agglutination reaction. If, for example, the blood agglutinates in the presence of anti-A and anti-Rh serum, the blood sample is determined a type A+. If none of the samples agglutinate, the blood type is O-.

Table 3: Blood Type/Antisera Reactions

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

Rh Factor and Pregnancy

The fact that there are 26 different blood typing systems makes things complicated enough. To further complicate matters, even within these various systems, there may be multiple antigens. For example, in the Rh system there are over 45 different identified antigens. Fortunately, many of these antigens are either very rare or very weak, failing to stimulate an immune reaction. There is one, called the D antigen (originally classified by Wiener as the Rho antigen), that is quite strong and will elicit an immune response. Like ABO types, this can greatly affect the safety of blood transfusions but there is another cause for concern with this particular Rh antigen, pregnancy.

As blood type is an inherited characteristic, it may be possible for a mother to be carrying a fetus of Rh+ even though the mother is Rh-. The Rh+ allele may have been inherited from the father. Usually, this is not a cause for concern, as fetal blood does not tend to mix with that of the mother. In some cases, however, there might be small leakages with blood from the fetus mixing with that of the mother. If the mother is Rh- and encounters Rh+ blood, the mother's immune system will recognize the antigens on these blood cells as foreign invaders and begin to manufacture antibodies against the Rh+ blood cells. Unfortunately, these antibodies may also recognize the Rh+ fetus as a foreign invader, essentially attacking the fetus' blood cells in an attempt to destroy them.

Rh factor is usually not an issue for a first pregnancy. In most cases, exposure to Rh antigens does not occur until late in a pregnancy, as the placenta begins to break down. If the mother is exposed to these antigens and begins to produce antibodies, she is said to be "sensitized" and continues to produce the antibodies even after the pregnancy. This can be of serious consequence in subsequent pregnancies that involve the presence of an Rh+ fetus.

Fortunately the situation is not as negative as it initially sounds. Before a mother becomes sensitized, she can be administered with an injection of Rh immunoglobulin (also called RhoGAM) which is a substance that prevents a reaction to the Rh antigen. Usually this will be administered about ⅔ through the pregnancy as well as shortly before birth, to ensure the mother does not become sensitized during the birthing procedure, helping to prevent complications in any potential future pregnancies.

Objectives

- Learn the role of antigens and antibodies in an immune response.
- Perform the blood typing procedure on four unknown blood donors.
- Examine the unknown blood samples for potential agglutination reactions.
- Determine the ABO/Rh blood type of each of the four unknown samples.
- Understand the importance of blood type to medicine.

Materials Included in the Kit

4	Simulated Blood Samples:
	Donor #1
	Donor #2
	Donor #3
	Donor #4
1 btl	Simulated anti-A serum
1 btl	Simulated anti-B serum
1 btl	Simulated anti-Rh serum
40	Blood typing trays
1pkg	Toothpicks

Safety

Goggles
Gloves
Aprons

Note to Instructor*: The simulated blood used in this activity is non-hazardous and contains no biological components. You may, however, wish to have students wear proper protective equipment to simulate the experience of doing real blood work.*

Procedure

Materials Needed per Group

4	Blood typing trays
12	Toothpicks

Shared Materials

Donor #1 simulated blood sample
Donor #2 simulated blood sample
Donor #3 simulated blood sample
Donor #4 simulated blood sample
Simulated anti-A serum
Simulated anti-B serum
Simulated anti-Rh serum

Safety (per student)

Goggles
Gloves
Apron

1. Using a wax marking pencil or a small piece of tape and a marker, label each of the four blood typing trays as follows: "#1." "#2," "#3," and "#4."
2. Add 3 drops of the blood labeled "Donor #1" to each of the three wells (A, B, and Rh) of the blood typing slide labeled "#1."
3. Add 3 drops of the blood labeled "Donor #2" to each of the three wells (A, B, and Rh) of the blood typing slide labeled "#2."
4. Add 3 drops of the blood labeled "Donor #3" to each of the three wells (A, B, and Rh) of the blood typing slide labeled "#3."
5. Add 3 drops of the blood labeled "Donor #4" to each of the three wells (A, B, and Rh) of the blood typing slide labeled "#4."
6. Place 3 drops of the anti-A serum in each of the wells labeled "A" on all four blood typing trays.
7. Place 3 drops of the anti-B serum in each of the wells labeled "B" on all four blood typing trays.

8. Place 3 drops of the anti-Rh serum in each of the wells labeled “Rh” on all four blood typing trays.

9. Using a toothpick, mix the contents of the well marked “A” of the blood typing slide labeled “#1” for 10-15 seconds. Using a new toothpick for each, mix the contents of the well marked “B” for 10-15 seconds and then the well marked “Rh” for 10-15 seconds.

10. Repeat the mixing procedure for the remaining trays (#2, #3, and #4). Be sure to use a new toothpick for each well.

11. Observe the wells of each blood typing tray for agglutination. Record your results in the Data Analysis section. Use a “+” if you see agglutination and a “–“ if there is no reaction.

12. Dispose of all materials according to your instructor and wash your hands before leaving the lab.

Note to Instructor: *The simulated blood is non-hazardous and can be washed down the drain with water. You may wish to have students wash their blood typing trays and return them for future use.*

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Table 1: Agglutination Reaction for Donor Blood Samples

	Anti-A Reaction	Anti-B Reaction	Anti-Rh- Reaction
Donor #1	(+)	(-)	(+)
Donor #2	(-)	(+)	(-)
Donor #3	(+)	(+)	(+)
Donor #4	(-)	(-)	(-)

Table 2: Blood Type of Donor Blood Samples

	Blood Type
Donor #1	A+
Donor #2	B-
Donor #3	AB+
Donor #4	O-

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

Questions

1. Based on your results, what antigens do you think Donor #2 has on their red blood cells? Why?

Based on the results, Donor #2 must have B antigens. The fact that there was agglutination in the X well means that antibodies in the anti-B serum must have been reacting with the B antigens on the surface of the red blood cells from Donor #2.

2. What do you think would happen if you mixed the blood from Donor #1 with Donor #2? Explain you answer.

If the samples were mixed, they would agglutinate. The anti-B antibodies from the blood plasma of Donor #1 would react with B antigens on Donor #2's blood cells. Conversely, the anti-A antibodies from the blood plasma of Donor #2 would also react with A antigens on Donor #1's blood cells.

3. When someone donates blood, the blood is mixed with a form of chemical called an anticoagulant. What do you think an anticoagulant is and how is it helpful to the storage of blood for future use?

In the case of a cut or similar injury, blood will clot to prevent the loss of further blood. Blood removed from the body will also clot. An anticoagulant is a chemical substance that prevents blood from clotting after it is removed from the body. The use of anticoagulants means blood can be collected and stored long-term for future use.

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

4. Usually before blood from one donor is transfused into a recipient, medical personnel will request a procedure called cross-matching be performed. Research and explain cross-matching and why it is a helpful procedure.

Cross-matching is a procedure in which red blood cells from the donor sample are mixed with blood plasma from the recipient. This test helps medical personnel determine if there may or may not be a reaction after blood transfusion and reduce the chance of further medical complications.

5. Besides blood type incompatibility, what other precautions do you think must be taken prior to the transfusion of blood?

Aside from incompatibility issues, donor blood must also be screened for any potential blood-borne pathogens, such as bacterial or viral infections. While a blood transfusion may be necessary to save someone's life, precautions must be taken to ensure the patient is not being exposed to any other risks.

6. In very recent research, scientists claim to have discovered certain enzymes that can be of great benefit to blood centers and the medical community. Using any resource available, research the use of these enzymes and explain how they could help make donated blood safer for use.

A group of scientists have recently revealed a study in which they used select enzymes to remove the antigens from the surface of red blood cells. Essentially, the blood cells are converted to type O blood, which is the universal donor. Blood type O is the most used, and therefore shortest in supply for transfusions. Converting all donated blood to blood type O would be of tremendous benefit to medicine.