Forensic Chemistry of Blood Typing
IS 9008
Student Guide

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 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 of absence of these molecules. Blood cells containing only the A molecule, Landsteiner called blood type A, on blood cells with only the B molecule, he called blood type B, on 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	A, B, AB, O
O	A, B, AB, 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 antigens 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 are 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	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)

ABO Blood Types and Genetics

Like so many other traits of an individual, such as eye color for example, blood type is an inherited characteristic. That is to say that the likelihood of an individual having a certain blood type is dictated by both the paternal and maternal donor's blood types.

In his famous experiments involving certain traits in pea plants during the 1800s, Gregor Mendel, an Austrian monk, showed that the probability of a certain characteristic being present in a pea plant was determined by the characteristics present in the plants that were crossed to produce the seed for the new plant. The reason, Mendel eventually concluded, was that that these traits were controlled by what are called genes. The way these genes express themselves in an individual (whether it be a human or pea plant) is determined by a pair of genetic fragments, called alleles, with one allele being inherited from each parent.

In order to fully understand the method of inheritance, some genetic terms must first be understood: phenotype, genotype, dominant, recessive, homozygous, and heterozygous. Using eye color as an example, these terms can be illustrated.

Eye color is an inherited characteristic, again, determined by the pair of eye color alleles inherited, one paternally and one maternally. Though a variety of eye colors may be present in humans, for simplicity's sake we will assume only two eye colors exist, brown and blue. An offspring's eye color is the result of the pair of inherited alleles. An individual may inherit two alleles for the same color eyes (e.g. two alleles for brown eyes). This individual is said to be **homozygous**. The offspring may have two different eye color alleles (e.g. one allele for brown eyes and one allele for blue eyes). This person is **heterozygous.**

In the case of many inherited traits, one of the possible characteristics is usually "stronger", or more likely to express itself. This trait is called the dominant trait. The trait less likely to express itself is called the recessive trait. In the case of eye color, brown is the dominant trait. When examining genetic traits, the alleles are usually denoted as a letter, with the dominant version being capitalized and with the recessive version a lowercase of the same letter. In the example of eye color, the allele for brown eyes (dominant) is B and the allele for blue eyes (recessive) is b.

Since there are only two possible alleles, coming from two different sources, there are three possible combinations of inherited alleles: BB, Bb, and bb. The combination of alleles is referred to as **genotype**. The way the combinations express themselves in an individual is referred to as **phenotype**. It is very important to understand the difference between these two terms so to re-state, phenotype is the physical expression of an allele combination and genotype is the actual allele combination, regardless of expression. Since one of these alleles is dominant (brown), there are only two possible phenotypes (brown eyes or blue eyes) even though there are three possible genotypes (BB, Bb, bb). Therefore, an individual with either the genotype BB (homozygous, dominant form) or Bb (heterozygous) will have brown eyes. The only combination that can lead to blue eyes is bb (homozygous, recessive form).

Understanding these terms and concepts, one can also begin to understand the inheritance of blood types. There is one important difference with blood types but the mode of inheritance is the same, with one allele for blood type coming from each parent. In the case of blood types, there are three possible alleles, one for type A blood, one for type B blood, and one for type O blood. The allele for type O blood is always recessive to either the type A or type B alleles. However, in some cases, two alleles may both fully express themselves phenotypically. This condition is called **codominance** since both alleles are acting as dominant traits. This is manifest in the case of blood type AB, since an individual with blood type AB must have one type A allele and one type B allele And neither is dominating the other, they are both fully expressing in the individual.

Alleles for blood type are usually presented in the following manner: I^A (type A), I^B (type B), and I^O (type O). The following is a chart of all possible phenotypes and genotypes for the ABO blood types:

Blood Phenotype	Possible Genoytpes
Type A	I^AI^A, I^AI^O
Type B	I^BI^B, I^BI^O
Type AB	I^AI^B
Type O	I^OI^O

Forensics and Blood Typing

Knowledge of blood types and blood typing has served many important purposes. One of the more interesting applications has been its use in a variety of criminal investigations. Since blood type is inherited, it has been used in paternity disputes and suspected kidnapping cases. Suppose an infant is kidnapped from a hospital. The police may have a couple suspected of kidnapping the infant. The couple may claim the child is theirs. By taking the blood types of the parents of the missing child, the parents suspected of kidnapping the infant, and the blood type of the infant, police investigators and scientists can statistically prove the likelihood that the child could have been the offspring of either set of parents, and, in some cases, almost completely eliminate the possibility that one couple may have parented the child.

Another example may be in the case of blood found at a crime scene. Imagine returning home to find your house has been burglarized. A window is broken and several items are missing. Police investigators find blood on the broken glass of the window. Apparently, the perpetrator cut themselves during the commission of the crime. After apprehending possible suspects, the investigators can determine not only the blood type of the sample at the crime scene, but also the blood types of the suspects. Using this information, investigators may be able to eliminate certain suspects from their investigation. Blood type alone may be enough to eliminate a suspect but since so many individuals have the same blood type it will not be enough to prove guilt. However, when combined with other traces of evidence, blood type may help in building a case against a suspect.

Scenario

A man placed a call to the police. He had been sitting at home and heard the sound of breaking glass in another room. When entering the room from which he had heard the noise, he noticed a figure in the dark. Realizing someone was attempting to rob him, the man engaged in a struggle with the perpetrator. After a short while the perpetrator fled. During the struggle, the man was pretty sure he injured the perpetrator. As it was dark in the room, he could not be sure. Also, since it was dark, he did not get a good look at the person.

When the police arrived, they discovered not only a broken window in the room but blood on the floor where the struggle occurred. Meanwhile, police patrols that were dispatched to the neighborhood after the call located two suspicious characters in the area. Both were taken into custody. Investigators at the scene of the crime collected a sample of the blood found there and sent it off to the lab.

The sample was sent to you and your fellow lab technicians, as well as blood samples from both suspects. Also, blood was taken from the victim to determine whether or not the blood at the scene could be his, either resulting from the struggle or a previous accident. Your job is to determine all the blood types of the samples and see if they offer any help to investigators in either further supporting the likelihood of one of the suspect's guilt or exonerating a suspect.

Materials Needed per Group

4 Blood typing trays
12 mixing sticks
Wax marking pencil or marker

Shared Materials

Victim blood sample
Suspect #1 blood sample
Suspect #2 blood sample
Crime scene blood sample
Anti-A blood serum
Anti-B blood serum
Anti-Rh blood serum

Objectives

- Understand the difference between ABO/Rh blood types.
- Perform ABO/Rh blood typing on 4 different individuals.
- Determine likelihood of match between unknown blood sample and two suspects in a criminal investigation.

Procedure

1. Using a marker or wax pencil, label each of your 4 blood typing trays as follows: "Victim", Suspect #1", "Suspect #2", and "Crime Scene."

2. Place 3-4 drops of blood from the sample labeled "Victim" in each of the A, B, and Rh wells of the blood typing tray labeled "Victim."

3. Add 3-4 drops of anti-A blood serum to the A well on the tray.

4. Add 3-4 drops of anti-B blood serum to the B well on the tray.

5. Add 3-4 drops of anti-Rh blood serum to the Rh well on the tray.

6. Using three mixing sticks, gently stir the mixtures of blood and anti-sera together in all three wells. Be sure to use a different mixing stick for each well to avoid cross-contamination.

7. Examine each of the wells for agglutination. Record your results in the Data Analysis sheet.

8. Based on the presence or absence of agglutination, determine the victim's blood type and record it in the Data Analysis sheet.

9. Repeat steps 2-8 for both of the suspects' blood samples and the crime scene blood sample. Be sure to use the properly labeled tray for each sample. Record your results for each in the Data Analysis sheet.

10. Write a brief paragraph in the "Conclusion" section of the Data Analysis sheet for the police investigators. Be sure to address the following in your summary:

    - Could the sample have come from either suspect?
    - Can either suspect be eliminated from suspicion based on the results?
    - Could the sample at the crime scene have come from the victim?

Name:	Instructor:
Date:	Class/Lab Section:

DATA ANALYSIS

	Victim	Suspect 1	Suspect 2	Crime Scene
Anti-A (+/-)				
Anti-B (+/-)				
Anti-Rh (+/-)				

Conclusions