ImBT Blood Typing

Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Explain how immunological tests can be used to identify microbes and determine a person's blood type.
- Use agglutination and ELISA to detect the presence of specific proteins in a sample.

Background/Theory

Scientists can take advantage of antibody specificity in diagnostic testing and research. Because the variable region will bind to only one epitope, antibodies can be used as probes for very specific organisms or antigens in a mixture. For example, if a bacterium displays the epitope that interacts with an IgM binding site, you can imagine up to 10 bacterial cells "sticking" to a single IgM. When each of these bacteria bind to other homologous IgM's and those IgM's complex with additional target cells, a large aggregate forms. This clumping is called **agglutination**. To be used in a test, these large aggregates must be observable. In **direct agglutination**, the antigen is naturally large enough that the resulting aggregate is visible to the human eye.

Blood typing is an excellent example of a direct agglutination reaction. Red blood cells are coated in antigens made of glycolipids and glycoproteins. In humans, surface antigens are grouped into 24 different blood groups with more than 100 different antigens on each red blood cell. (OpenStax Biology, 2018) The best known blood groups are the ABO system and the Rh system.

In the **ABO system**, two different carbohydrate groups can be added to a protein receptor molecule present on all red blood cells. People with the carbohydrate antigen A have type A blood. Those with carbohydrate B have type B blood. Those with both types of carbohydrate antigens are type AB. People with neither group attached to the protein receptor have type O blood.

To test for each of these antigens, commercially prepared antisera are used. Anti-A contains IgM that will react with the A antigen resulting in agglutination. If the B antigen is present, Anti-B serum will agglutinate the blood. If both antigens are present, agglutination will be observed when mixed with anti-A and when mixed with anti-B. If the protein receptor has neither antigen, neither anti-serum will react.

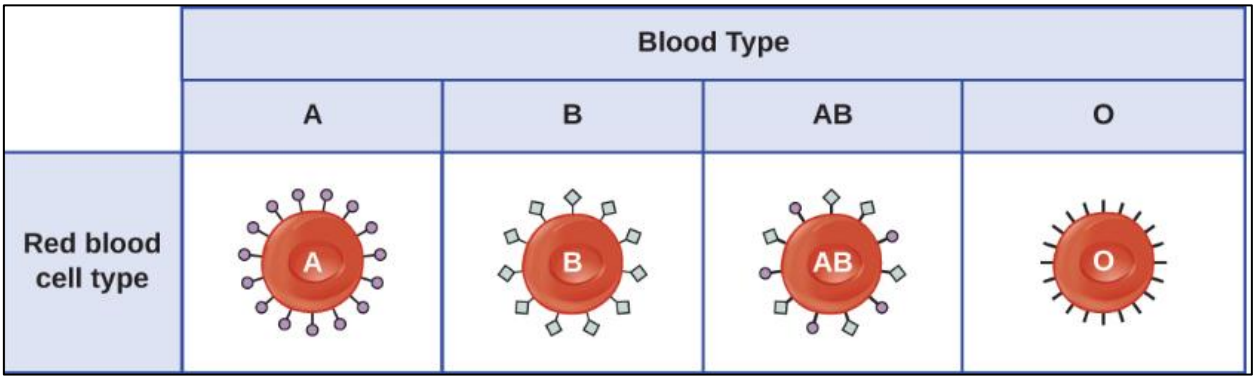

Figure 1 ABO Blood Types and their antigens (OpenStax CNX, 2018)

The **Rh blood group** was first discovered in Rhesus monkeys. In contrast to the carbohydrate molecules that distinguish the ABO blood groups and are the targets of IgM, the Rh factor antigens are proteins. These protein antigens induce a different immune response. Protein antigens activate B cells and antibody production through a T-cell–dependent mechanism, and the T-cells stimulate class switching from IgM to other antibody classes. In the case of Rh factor antigens, T-cells stimulate class switching to IgG. (OpenStax CNX, 2018)

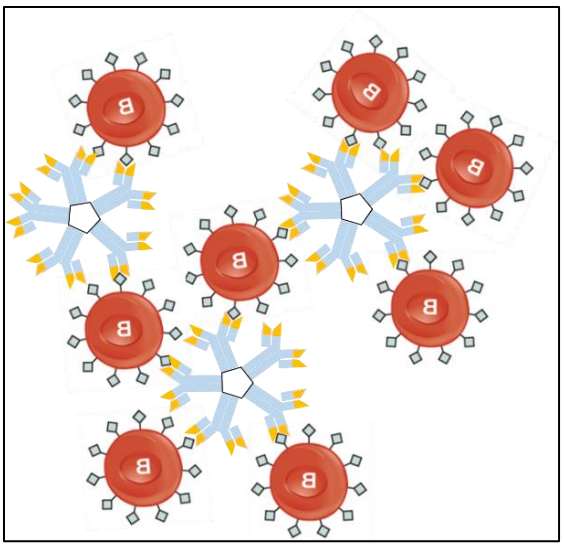

Figure 1 Hemagglutination. Type B red blood cells reacting with anti-B IgM. Adapted from (OpenStax CNX, 2018)

Most people have the Rh antigen (Rh+) and do not have anti-Rh antibodies in their blood. The few people who do not have the Rh antigen (Rh–) can develop anti-Rh antibodies if exposed to Rh+ blood. This can happen after a blood transfusion or after an Rh– woman has an Rh+ baby. The first exposure does not usually cause a reaction; however, at the second exposure, enough antibodies have built up in the blood to produce a reaction that causes agglutination and breakdown of red blood cells. An injection can prevent this reaction. (OpenStax Biology, 2018) This is called **hemolytic disease of the newborn** (OpenStax CNX, 2018) , more commonly referred to as **maternal-fetal Rh incompatibility**. The presence of Rh antigen is determined by agglutination when anti-Rh sera is added to a drop of blood.

Experiment/Exercise

Materials at each station

Paper towels or scratch paper
Safety lancets
Alcohol wipes
Anti-sera: Anti-A, Anti-B and Anti-Rh
Reaction cards
Clean or sterile toothpicks
Band-Aids
Gloves
Sharps disposal container
Other disposal container

Cultures

None

Procedure Lab 1

1. Read this entire procedure before beginning and follow it carefully. The order of each step is important and necessary for your safety.
2. <u>You and only you</u> are to handle your own blood. You must be sure to <u>clean up after yourself</u>!
3. Remove your gloves and dispose of them in the disposal container for gloves.
4. Prepare your materials
   a. Lay out two paper towels on the bench top. Set the reaction card in the center.

   b. Place a new pair of gloves, an unwrapped bandage and 2 opened alcohol wipes on the paper towel so that they are ready to be used.
5. Decide which finger you will prick and shake the blood down into your fingertips. Swab the finger with the alcohol wipe. Place the used wipe in the disposal container immediately.
6. Remove the tab from the safety lancet, place the tip against the side of your finger and push the barrel of the lancet to discharge it. Prick the SIDE of your finger where the epidermis is thin. Avoid the tip/pad of your finger.
7. Place a drop of blood into each of the spaces on the card designated A, B and Rh.
8. Quickly wipe your finger once more with the second alcohol pad disposing of it in the container immediately. Place the bandage on your finger. Put the new pair of gloves on.
9. Next to each drop of blood, but not touching it, place a drop of each of the Anti-sera.
10. With a tooth pick mix each drop with the antisera. Be sure to use a new toothpick for each antisera and place the toothpick in the disposal container immediately after use. Do not set the tooth pick down anywhere on the bench or paper towel.
11. Rock the card back and forth to further mix the blood with each antisera. Take care not to allow the mixture from one reaction circle to contact a different mixture.
12. Record agglutination in the data table.
13. Clean up.
    a. Place the card on the paper towel along with any wrappers. Carefully fold up the paper towel and place all the waste in the disposal container.
    b. Spray the work area with disinfectant and wipe up with a paper as you do at your bench at the end of the lab session.
    c. Dispose of your gloves and get another pair.
14. Determine your blood type based on which antigens are present on your red blood cells.

ImBT

blank

Lab Report: Immunology Overview/Blood Typing

Name _____
Lab Section _____

Data and Observations

Antiserum	Agglutination +/-	Antigen present on RBCs		Blood type

Post Lab Questions

1. Which region of the Ig molecule interacts with the antigen? What makes this region suited for this purpose?

2. Which Ig class is involved in this particular agglutination reaction?

3. Fill in the following table.

Blood type	Antigens present on RBCs	Anti-IgM(s) causing agglutination	Compatible blood types for a donor
B+			
O-			
AB+			

4. Read about Rh incompatibility here or scan the QR code.
   a. With respect to a blood transfusion, under what conditions is Rh incompatibility a problem? List the recipient blood type and the donor blood type.

   b. During pregnancy, under what conditions is Rh incompatibility a problem? List the mother's blood type and the fetus blood type. In which pregnancy?

   c. Explain the role that the Ig class plays in maternal-fetal incompatibility? Hint: What class is involved? Then consider the longevity of the antibody class in the mother's circulation.

   d. Explain why this is an issue usually in the second pregnancy?

5. There is a lesser known phenomenon of maternal-fetal ABO incompatibility. Why is this much less common than Rh incompatibility? (Hint: think about the Ig classes involved in each.)

References

OpenStax Biology. (2018, Sep 26). OpenStax Biology 2nd Edition, Biology 2e. Retrieved from http://cnx.org/contents/8d50a0af-948b-4204-a71d-4826cba765b8@14.24

OpenStax CNX. (2018, Mar 19). OpenStax Microbiology. Retrieved from http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.24

ImBT

blank