

BLOOD TYPING

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I. OBJECTIVES:

1. To study blood types as related to human genetics.
2. To learn how blood types are determined.

II. BACKGROUND:

Our blood cells have a variety of chemicals on their surfaces called **antigens** (derived from **antibody generating**). Blood cells from different people have different antigens (**anti** = against, opposite) on them, and which ones a person has depend on what genes that person has. A person cannot develop antibodies against his/her own antigens – one job of the immune system is to be able to tell “me” from an “invader.” Our immune systems can make substances called antibodies that fight against specific antigens. This is useful in fighting off invading cells, but means that a person could develop an allergic-type reaction if exposed to blood cells from another person with a different blood type.

If you ever need to receive blood, it is important that you receive the proper type of blood. The wrong type could trigger an immune response and cause **agglutination** (**agglutin** = glued together), or clumping, of the red blood cells (**RBCs**). Blood can be typed by mixing it with various antisera that mimic this clumping behavior, indicating the presence of the antigen being tested. Agglutination with an antiserum thus indicates that you have that blood type while lack of agglutination indicates a lack of that particular antigen.

If a person with type A blood comes into contact with type B blood, that person will develop antibodies to type B and would have a severe reaction to any further attempts to give a type B transfusion. The opposite is true for type B blood. People with type AB blood will not develop antibodies to either A or B antigens and thus could receive any kind of blood (ABO blood group) as a transfusion. Type O blood can develop antibodies to both A and B antigens and so can receive only type O blood. However, type O blood will not cause types A or B blood to form antibodies because it has no antigens. Thus, in an emergency, type O can be used as the “universal donor” (using the same type is still preferred and safest) while type AB is referred to as the “universal recipient.”

Another “problem” involving blood types is that of **Rh** or **Rhesus** factor, so named because first discovered in *Rhesus* monkeys. The problem arises when an Rh⁻ mother has an Rh⁺ baby. The first baby is usually not affected, but if, during birth, any of the baby’s

blood comes into contact with the mother’s, she could develop anti-Rh⁺ antibodies which could “attack” the blood of subsequent Rh⁺ babies. Note that Rh⁺ indicates the presence of the Rh antigen and Rh⁻ indicates a lack of this particular antigen. Because of potential Rh incompatibility, it is suggested to all first-time expectant parents that they have their blood typed. Postpartum shots (Rho-Gam) are now available which trick the new mother’s immune system into “thinking” that she already is producing anti-Rh⁺ antibodies so production of these antibodies never starts. Also, an Rh⁻ woman who experiences a miscarriage should have a Rho-Gam shot when she goes to the hospital. Note that an abortion, especially of the type in which the baby is pulled to pieces, would be more likely to cause the mother’s blood to be exposed to that of her baby with possible subsequent Rh incompatibility than a regular birth, especially since the instruments used to remove the baby also frequently cause small to large lacerations on the woman’s cervix and/or uterus. Since Rh⁺ is the presence of the Rh factor and Rh⁻ is the absence of that factor, Rh⁻ blood can develop anti-Rh⁺ antibodies, but Rh⁺ blood which has the factor cannot develop antibodies against Rh or it would destroy itself. Thus, Rh⁺ can accept Rh⁻ blood, but Rh⁻ cannot accept Rh⁺.

The human ABO blood group is an example of **multiple alleles** for one gene. With eye color in humans, a person can be BB, Bb, or bb as brown or blue are the only two choices for this gene. For the ABO blood group gene, however, there are more choices. There are three possible alleles at this gene site, namely I^A, I^B, and i. Thus, a person could be I^AI^A or I^Ai and have type A blood, I^BI^B or I^Bi and have type B blood, I^AI^B and have type AB blood, or ii and have type O. One allele is inherited from each parent, thus, a baby’s blood type is used as court evidence in paternity suits. About 45% of the population of the U. S. is type O, 42% type A, 10% type B, and 3% type AB. About 85% of the population is Rh⁺ and 15% Rh⁻. Thus, a person’s chances of being, for example, O⁻ would be 45% × 15% or 6.75%. The rarest blood type in the U. S. would be AB⁻ which would be only about 0.45% of the population. These various alleles are not evenly distributed among all humans. In some cultures, 100% of

the people have type O blood, while other cultures exist where 75% of the people have type A and 25 have type O (no B or AB) or where 40% of the people have type B (with a mixture of the other types).

There are other blood factors too, such as the MN group in which a person can be type M, type N, or type MN. Other antisera are available to test for this blood group. Note

III. SAFETY CONSIDERATIONS:

In this lab exercise, we will be working with human blood, thus a number of safety precautions must be observed to prevent the spread of blood-borne pathogens. THINK before you act, use common sense, and do not do anything that could bring you into direct contact with someone else’s blood or risk exposing anyone else to your blood. Students who are behaving in an unsafe manner will be asked to leave the lab, and if needed, the security guard will be asked to escort such persons out of the area. You absolutely, positively **MUST!!! CLEAN UP** after yourself, down to the last toothpick! All soiled items must be properly disposed of in the designated locations. Make sure you observe the following precautions:

1. **ABSOLUTELY NO FOOD OR DRINKS SHOULD BE CONSUMED WHILE THIS LAB IS BEING PERFORMED AND/OR UNTIL THE LAB ROOM IS TOTALLY FREE OF ANY BLOOD-STAINED ITEMS AND THE TABLE TOPS HAVE BEEN DECONTAMINATED.**

2. Keep your hands out of your eyes and mouth.

3. Wear gloves if you need to help someone else.

4. Use only a sterile lancet (if it has been sitting out on the desk top – which it shouldn’t be – it’s no longer sterile, and might be contaminated with someone else’s blood) on your finger. Do not set a lancet without its cap, loose, on the desk top – it will become contaminated with air-borne bacteria or someone else’s blood. Also, do not attempt to recap a used lancet. Use only clean toothpicks and Kimwipes or cotton balls.

5. When you are finished with the

also that Rh factor is actually also a multiple allele blood group like the ABO blood group. The most common allele for Rh factor is the D allele, and this is the only one which is commonly tested. Although comparatively rare, there are other alleles and a person who tests negative to the D allele could possibly have Rh⁺ blood to one of the other rarer alleles.

lancet, immediately put it in the hard-walled **sharps container**. THIS IS THE **ONLY** PLACE WHERE USED LANCETS SHOULD BE PLACED, and **ONLY** “sharps” should be placed in the sharps container.

6. Any blood-stained or potentially blood-stained disposables such as lancet caps, toothpicks, Kimwipes, and/or cotton balls must immediately be placed in the designated location so that they may be disinfected by lab personnel. Do NOT let any toothpicks drop on the floor – you will be using three, so you should be disposing of three (if needed, count them as you dispose of them). **NONE OF THESE ITEMS SHOULD BE PLACED IN THE REGULAR TRASH!**

7. Blood-stained, glass microscope slides should NOT be cleaned off at the sink. They should be placed “as is” into a 10% solution of Clorox in the designated dishpan. Gently place the slides into the solution so that no splashing occurs (goggles to prevent eye damage are strongly recommended). Do not let your fingers come into contact with the solution. 10% Clorox is supposed to be effective at disinfecting blood products, which is why it is being used. When all the slides are collected, lab personnel will see that further steps are taken to insure proper decontamination.

8. If any blood drops anywhere, wipe up the spill with a Kimwipe or paper towel dipped in 10% Clorox-water to disinfect the area, then place the paper in with the waste to be disinfected. If the spill is large, immediately notify your instructor and ask for help, because the area must be treated with 10% Clorox-water to disinfect it.

IV. MATERIALS NEEDED:

clean microscope slide

wax pencil

lancet

anti-A, anti-B, and anti-D (Rh) sera

70% alcohol (EtOH) and cotton or Kimwipe

3 toothpicks

Rh viewbox

V. PROCEDURE:

First, a note of caution: For many, many years countless technicians have safely performed blood typing on patients. With the recent epidemics of the AIDS and hepatitis viruses, however, someone might question the safety of doing this lab. If you exercise common sense and caution, this will not be a problem. Do not do anything that would cause someone else's blood to enter your body (like stick yourself with a used lancet) nor anything that would risk exposing anyone else to your blood. To avoid exposure to any blood-borne pathogens, each person should lance his/her own finger. If, for some reason, it becomes necessary to help someone else, wear gloves. Also due to Federal health/safety regulations, **NO BLOOD OR BLOOD-STAINED ITEMS SHOULD BE PUT INTO THE TRASH CAN OR DOWN THE DRAIN** – this could endanger people and/or cause legal problems for the school. A special container has been designated for disposal of lancets (and other "sharps"). **Toothpicks, cotton, etc. should be placed in the specially designated container to be decontaminated.** Do not leave these items lying around the lab – **YOU MUST CLEAN UP YOUR OWN MATERIALS!!!** Yes, you know your blood is safe, but the lab staff and maintenance people that clean up the lab won't know if it came from you or someone else.

Before performing this lab exercise, review safety issues and proper procedure by viewing and becoming familiar with the information presented on the Web page <http://biology.clc.uc.edu/courses/bio112/blood.htm>.

1. Obtain a microscope slide. If it is not clean, clean it. The slide must be very clean so it does not interfere with the reaction.

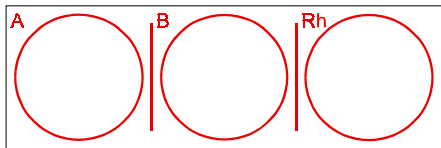


Figure 1. Wax Circles

2. With a wax pencil, draw two lines on one surface of the slide to divide the surface into thirds (do NOT peel the wax pencils just to play with them). In each third, draw a large circle that fills the space. Label the circles "A," "B," and "D" or "Rh" in the corner OUTSIDE the circles (Figure 1). Also, put your initials on a corner of your slide.

3. Obtain three toothpicks (one for each circle) and place near the circle for which each will be used. Obtain an Autolet®, load a lancet into it, and place an orange finger platform on it.

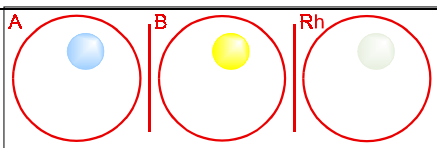


Figure 2. Antisera Added

4. Place one drop of the appropriate antiserum (at room temperature) near the edge, but within each of the circles. Be very careful – be sure you do NOT cross contaminate the sera! Do NOT let them touch or mix (the wax pencil rings should help to contain them). Do NOT let the droppers get mixed up.

5. Choose a little-used finger (usually left ring finger). Clean this fingertip with alcohol on a cotton ball or Kimwipe, and let it air dry. Keep the cotton ball/Kimwipe nearby and clean – you'll need it again. Dangle the hand down to increase the amount of blood in the fingers (don't touch anything with the clean finger). Press on the "bottom" of the fingertip with the thumb of the same hand (to help hold blood in the fingertip) and with a quick, deep jab (press the orange platform tightly against your finger), lance the "fleshy" part of the side of the fingertip on the "little-finger side" of the tip. Note that the lancet is sterile when you remove its cap, so do not touch the tip with anything before using it.

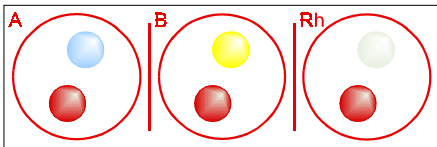


Figure 3. Blood Added

6. Working quickly, let one drop of blood drop into each circle but not touching the antisera yet. Do not get any antiserum on your finger. Keep your arm below your heart and your hand below your elbow, and massage the hand and/or finger towards the tip to get more blood if needed. When you have your three drops of blood, you may then apply gentle pressure to the wound with your cotton ball/Kimwipe (use a clean one if you suspect that your previous one may have become contaminated with someone else's blood) to stop the bleeding. Note that the blood will flow better if the hand is held down (below the heart) rather than up. **REMEMBER TO PROPERLY DISPOSE OF THE LANCET BEFORE SOMEONE ELSE GETS STUCK WITH IT.** The cap does not need to go into the "sharps" container – only the lancet.

7. Using one toothpick for each circle, stir the blood and antiserum until homogeneous, but do not overmix. Do this for each of the three. Place on the Rh viewbox which will also warm the slide. The wax pencil circles will help to keep the samples isolated and contained.

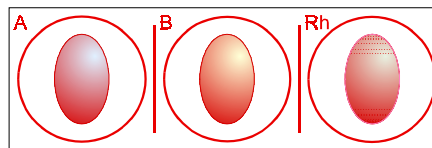


Figure 4. Stirred, Agglutination in Rh

8. Watch to see if any of the samples show agglutination. This would appear as grainy clumps of red blood cells (RBCs) suspended in a clear solution (as opposed to a generally red color). Rh is slower to agglutinate, so don't give up too soon. Reaction with a particular antiserum (= antibodies) indicates that you have that

VI. DATA:

Take notes on procedure, problems, etc. Record your blood type in your notebook and in the computer as directed. Copies of all information will then be provided so that you can calculate what percentages of the class are each of the blood types.

VI. DISCUSSION:

1. Now that you know your blood type, to whom (what blood type) can you give blood and from whom may you receive blood?

2. Do you know the blood types of any other members of your family? People who have been in the military have had their blood typed, as well as anyone who has ever given to a blood bank. If you can get enough information, try to figure out the Punnett square for your parents having you and your siblings and/or the Punnett square for you and your spouse/fiancé having children. ABO would be one gene and Rh would be another,

antigen on your RBCs – you have that blood type. If no agglutination appears in any of the samples, you have O⁻ blood.

9. Note any agglutination in your lab notebook. Place the slide in the designated dishpan of 10% Clorox for decontamination. Properly dispose of your cotton ball/Kimwipe and your 3 toothpicks in the designated container.

10. **MAKE SURE ALL BLOOD-STAINED ITEMS ARE DISPOSED OF PROPERLY.** Nothing should go in the regular trash, nothing should be left on the table tops, and nothing should be left on the floor.

thus each alone would be a monohybrid cross, but the two together would be a dihybrid cross.

3. For your blood type, figure out what percentage of the general population of the U. S. has the same ABO/Rh type as you. How do each of the percentages for the various blood types for the class compare to the percentages of those types for the general population? In one previous lab sections, everyone in the class had either type O⁺ or O⁻, a rather atypical distribution.