Blood Analysis and Hemostasis

Objective:

To explain in words or diagrams, the importance of hematocrit, hemoglobin levels, clotting time and blood typing, at the level of 85% proficiency for each student.

In order to achieve this objective, you will need to be able to:

1. Conduct the following blood tests: hematocrit, hemoglobin level, and ABO and RH blood typing and to list the norms and importance of each.
2. Explain the reason for transfusion reactions resulting from the administration of mismatched blood.

Materials:

General Supply:

Models and charts of blood cells
Clean microscope slides
Sterile safety lancets
Alcohol swabs (wipes) or cotton balls and isopropyl alcohol
Test tubes
Test tube racks
Disposable gloves
Large beaker containing 10% household bleach solution for disposal of slides
Large beaker containing 10% household bleach solution for disposal of glass tubes
Sharps container
Disposable biohazard bag
Spray bottles containing 10% bleach solution
Spray bottles containing 70% isopropyl alcohol
Because many blood tests are conducted in this exercise, separate supply areas are set up for the various tests

Hematocrit supply area:

Heparinized capillary tubes
Micro-hematocrit centrifuge and reading gauge (if the reading gauge is not available, millimeter ruler may be used)
Seal-ease (Clay Adams Co.) or modeling clay

Hemoglobin determination supply area:

Hemoglobin monitor

Blood typing supply area:

Blood typing sera (anti-A, anti-B, and anti-Rh [D])
Rh typing box
Wax marker
Toothpicks
Clean microscope slides

Common Methods:

In this lab Safety lancets are used to obtain blood samples.

- Students are permitted to handle only their own blood.
- "Finger-stick" devices (Safety lancets) must be placed in the Sharps Container after use.
- Cotton balls, and items exposed to blood must be placed in the Biohazard Bag

Safety concerns are outlined here:

- U.S. Food and Drug Administration https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/HomeHealthandConsumer/ConsumerProducts/Sharps/default.htm

Procedures for using safety lancets and obtaining blood samples will be described in class.

Accepted procedures for handling "finger-stick" devices (including safety lancets) and using them to obtain blood samples are outlined in:

- Lab_Appendix_A from my website https://www.dgward.com/pdf/physo101/Lab_Appendix_A_finger-stick.pdf

Hematocrit

Hematocrit is routinely determined when anemia is suspected. Centrifuging whole blood spins the blood cells to the bottom of the tube, with plasma forming the top layer. The percentage of the blood that is cells is called the hematocrit (Hct). Since over 99% of blood cells are RBCs, the hematocrit is a very accurate measure of RBCs. Normal hematocrit values for the male and female, respectively, are 47.0 ± 7 and 42.0 ± 5.

Methods:

The hematocrit is determined by the micro-method, so only a drop of blood is needed. If possible, all members of the class should prepare their capillary tubes at the same time so the centrifuge can be properly balanced and run only once.

1. Obtain one heparinized capillary tube, Seal-Ease^{tm} or modeling clay, a safety lancet, alcohol swabs, and some cotton balls.

2. Clean and prick the finger to produce a free flow of blood**.** Wipe away the first few drops and, holding the yellow-line-marked end of the capillary tube to the blood drop, allow the tube to fill at least three-fourths full by capillary action. If the blood is not flowing freely, the end of the capillary tube will not be completely submerged in the blood during filling, air will enter, and you will have to prepare another sample.

3. Plug the blood-containing end by pressing and rotating it into the Seal-Ease^{tm} or clay.

4. Place the prepared tubes opposite one another in the radial grooves of the micro-hematocrit centrifuge with the sealed ends abutting the rubber gasket at the centrifuge periphery. This loading procedure balances the centrifuge and prevents blood from spraying everywhere by centrifugal force. *Make a note* of *the number (location) of the groove your tube is in.* When all the tubes have been loaded, make sure the centrifuge is properly balanced, and secure the centrifuge cover. Turn the centrifuge on, and set the timer for 4 or 5 minutes.

5. Dispose of the safety lancet in the sharps container, the hematocrit tube in the bleach container, and soft supplies with blood in the biohazard bag.

Determine the hematocrit (HCT, percentage of RBCs) by using a micro-hematocrit reader. Place the hematocrit tube on the reader with the bottom of the cell layer on the zero (0) line. Move the tube left or right until the top of the plasma layer is on the 100% line. Find the line that intersects with the top of the cell layer and follow it to the left side of the graph to read the percentage of RBCs (HCT). The percentage of plasma is 100% - HCT.
If you have a small quantity of blood in the tube, use a millimeter ruler to measure the height of the **entire column** of blood and the height of the **portion with RBCs**. Compute the percentage of RBCs (HCT) by using the following formula:

$$\frac{\text{Height of the column composed of RBCs (mm)} \times 100}{\text{Height of the entire column of whole blood (mm)}}$$

The percentage of plasma is 100% - HCT.

Results:

Record your calculations below**.**

% RBC (HCT) _____ % plasma _____

As a rule, a hematocrit is considered a more accurate test for determining the RBC composition of the blood than the total RBC count. A hematocrit within the normal range generally indicates a normal RBC number, whereas an abnormally high or low hematocrit is cause for concern.

Hemoglobin Concentration

As noted earlier, a person can be anemic even with a normal RBC count. Since hemoglobin is the RBC protein responsible for oxygen transport, perhaps the most accurate way of measuring the oxygen-carrying capacity of the blood is to determine its hemoglobin content. Oxygen, which combines reversibly with the heme (iron-containing portion) of the hemoglobin molecule, is picked up by the blood cells in the lungs and released in the tissues. Thus, the more hemoglobin molecules the RBCs contain, the more oxygen they will be able to transport. Normal blood contains 12 to 16 g hemoglobin per 100 mL blood. Hemoglobin content in men is slightly higher (l4 to 18 g) than in women (12 to 16 g).

Several techniques have been developed to estimate the hemoglobin content of blood. We will use a modern hemoglobin monitor with included instructions.

Methods

Each subject will measure Blood Hemoglobin using a drop of blood from a finger prick and a blood hemoglobin monitor

An Alere HemoPoint H2 meter is used to measure blood hemoglobin. Excerpts from the user's manual are reproduced in:

- Lab_Appendix_C from my website https://www.dgward.com/pdf/physo101/ Lab_Alere_HemoPoint_H2.pdf

Dispose of the safety lancet in the sharps container, and the cuvette and soft supplies with blood in the biohazard bag.

Results and Discussion:

Record the hemoglobin as gm/100 mL

Hemoglobin _____ gm/100 mL

Generally speaking, the relationship between the hematocrit (HCT) **and** grams of hemoglobin per 100 mL blood is 3:1. This ratio is a critical value used in a blood work-up for evaluating the oxygen carrying capacity of blood.

Normally the number for hematocrit is about three (3) times larger than the number for hemoglobin.

How do your values for hematocrit (HCT) and grams of hemoglobin per 100 mL compare?

_____

Blood Typing

Blood typing is a system of blood classification based on the presence of specific glycoproteins on the outer surface of the RBC plasma membrane. Such proteins are called **antigens,** and are genetically determined. In many cases, these antigens are accompanied by plasma proteins, **antibodies that** react with RBCs bearing different antigens, causing them to be clumped, agglutinated, and eventually hemolyzed. It is because of this phenomenon that a person's blood must be carefully typed before a whole blood or packed cell transfusion.

Several blood typing systems exist, based on the various possible antigens, but the factors routinely typed for are antigens of the **ABO** and **Rh** blood groups which are most commonly involved in transfusion reactions. Other blood factors are not routinely typed for unless the individual will require multiple transfusions. The basis of the ABO typing is shown in the table below**.**

Table 1. Blood types, surface antigens, and plasma antibodies

Blood Type	*Frequency*	*Surface Antigens*	*Antibodies typically in Plasma*
A	40%	A	anti-B
B	11%	B	anti-A
AB	4%	AB	none
O	45%	none	anti-A and anti-B
Rh+ (+)	84%	Rh	none
Rh− (−)	16%	none	none (anti-Rh if exposed to Rh blood)

Individuals whose red blood cells carry the Rh antigen are Rh positive (approximately 85% of the U.S. population); those lacking the antigen are Rh negative. Unlike ABO blood groups neither the blood of the Rh-positive (Rh+ or +) nor Rh-negative (Rh− or −) individuals typically carry Rh antibodies. This is understandable in the case of the Rh-positive individual. However, Rh-negative persons who receive transfusions of Rh-positive blood become sensitized by the Rh antigens of the donor RBCs and their immune systems begin to produce Rh antibodies. On subsequent exposures to Rh-positive blood, typical transfusion reactions occur, resulting in the clumping and hemolysis of the donor blood cells.

Rh+ is often abbreviated simply as +
Rh− is often abbreviated simply as −

Methods:

1. Obtain two clean microscope slides, a wax marking pencil, anti-A, anti-B, and anti-Rh typing sera, toothpicks, safety lancets, and alcohol swabs.

2. Divide slide 1 into two halves. Label the lower left-hand corner "A" and the lower right-hand corner "B." Mark the bottom of slide 2 "Rh."

3. Place one drop of anti-A serum on the *left* side of slide 1. Place one drop of anti-B serum on the *right* side of slide 1. Place one drop of anti-Rh serum in the center of slide 2.

4. Cleanse your finger with an alcohol swab, pierce the finger with a lancet, and wipe away the first drop of blood. Place one drop on each side of slide 1 and a drop on slide 2. **Do not allow your finger to touch the drops of antibody**.

5. Quickly mix each blood-antiserum sample with a ***fresh*** toothpick.

6. Place the slides on the **light box** and rock gently back and forth. (More time is required for precise Rh typing than for ABO typing.)

7. After 2 minutes, observe all three blood samples for evidence of clumping. The agglutination that occurs in a positive test for the Rh factor is sometimes difficult to perceive. **Ask for help if unsure.** Record your observations in the chart**.**

8. Dispose of the safety lancet in the sharps container, slides in the bleach container, and toothpicks and soft supplies with blood in the biohazard bag.

Results:

Blood typing

Blood mixed with	Clumping Observed (Yes / No)
“A” antibody	
“B” antibody	
“Rh” antibody	

1. Explain what your AB results show.

   _____

2. Explain what your Rh results show.

   _____

3. What is your blood type?

   _____

Discussion:

1. How do your hematocrit values compare to typical percentages for RBCs and plasma?
2. How do your hemoglobin values compare to the typical values?
3. What is the relationship between hematocrit and hemoglobin?
4. Explain the significance of your blood type in receiving or donating blood.
5. Explain why a mother with Rho negative blood and a father with Rho positive blood need to be concerned about a pregnancy.