Blood Lab

SAFETY NOTES:

Students will wear gloves during the lab period.

ALL materials that have come in contact with bodily fluids must be disposed of in biohazard containers. We have 2 types:

1. Sharps containers (red hard plastic containers). Use these containers for items such as lancets, microscope slides, coverslips, capillary tubes, toothpicks, etc.

2. Bag waste (big red plastic bag). Use the bag for any fluid-stained non-sharp item, such as pipette tips, cuvettes, alcohol swabs, paper towels, gloves, etc.

REMEMBER: it takes a lot of energy to autoclave red bag / sharp items. If there's no contamination, use the trash!!! If you have a big piece of paper towel and only a small drop of blood on it, please simply tear off the blood stained piece and dispose the rest of the paper towel in the trash.

Work on paper towels for easy clean-up. Wipe down your work area with Vesphere when you are finished.

Your safety, as well as the safety of your peers, your instructor, our technicians and custodial staff depend on everyone's good lab habits.

ANY QUESTIONS about safety, please ASK!

1. HEMOGLOBIN AND HEMATOCRIT

MATERIALS:
1. Heparinized capillary tubes, clay capillary tube sealant (crit-o-seal), microcapillary centrifuge, hematocrit reader
2. Microscope
3. Sterile lancets and 70% alcohol for preparing fingertip blood.
4. Spectrophotometer and cuvettes
5. Container for disposal of blood-containing items

Almost all of the oxygen transported by the blood is carried within the red blood cells attached to hemoglobin. Measurements of the oxygen-carrying capacity of blood include the red blood cell count, hemoglobin concentration, and hematocrit. Anemia results when one or more of these measurements is abnormally low.

OBJECTIVES:

1. Describe the composition of blood.
2. Describe the composition of hemoglobin and explain how hemoglobin participates in oxygen transport.
3. Demonstrate the procedure for hematocrit measurements, and list the normal values for these measurements.
4. Explain how measurements of the oxygen-carrying capacity of blood can be used to diagnose anemia and polycythemia.

Background Information:

Each ventilation cycle delivers a fresh supply of oxygen to the alveoli of the lungs. The amount of oxygen that leaves the lungs dissolved in plasma is equal to 0.3 mL of oxygen per 100 mL of blood. The amount of oxygen leaving the lungs in whole blood, however, is equal to 20 mL of oxygen per 100 mL of blood. Most of the oxygen (19.7 mL oxygen per 100 mL blood), therefore, must be carried within the cellular elements of the blood. This oxygen is carried by **hemoglobin** molecules within the red blood cells. In this way the oxygen is transported to the body cells and used for aerobic respiration and the production of ATP.

Each hemoglobin molecule consists of two pairs of polypeptide chains (one pair called the *alpha chains* and one pair called the *beta chains*) and four disc-shaped organic groups called *heme groups.* Each heme group contains one central ferrous ion (*Fe2+)* capable of bonding with one molecule of oxygen. Thus, one molecule of hemoglobin can combine with four molecules of oxygen.

The hemoglobin within the red blood cells load up with oxygen in the capillaries of the lungs and unload oxygen in the tissue capillaries. In both cases, oxygen moves according to its diffusion gradient. Since red blood cells always respire anaerobically (so they do not consume the oxygen they carry), a maximum diffusion gradient for oxygen is maintained between the red blood cells and the tissues.

The **oxygen-carrying capacity** of the blood is dependent on the total number of red blood cells and, consequently, on the total amount of hemoglobin. The total number of red blood cells is dependent on a balance between the rates of red blood cell production and destruction. The rate of red blood cell production by the bone marrow is regulated by the hormone **erythropoietin,** secreted by the kidneys. Erythropoietin is secreted when blood oxygen levels fall, such as when traveling in high-altitude environments. The rate of renal erythropoietin secretion is, therefore, regulated by the oxygen requirements of the body.

Older red blood cells (those that are approximately 120 days old) are routinely destroyed by the action of phagocytic cells fixed to the sides of blood channels (sinusoids) by a meshwork (reticulum) of fibers. Located in the spleen, liver, and bone marrow, these fixed phagocytes compose the **reticuloendothelial system.** These reticuloendothelial cells digest the hemoglobin within the old red blood cells into the component pats of protein, iron, and the *heme* pigment. The protein is hydrolyzed and returned to the general amino acid pool of the body, the iron is recycled to the bone marrow, and the heme is changed into a new pigment called **bilirubin.**

Bilirubin is released into the blood by the reticuloendothelial cells, then picked up by the liver and secreted into the bile as bile pigment. An abnormal increase in the amount of bilirubin in the blood, due to an increased rate of red blood cell destruction, liver dysfunction, or bile duct obstruction, results in the condition known as *jaundice* (yellowing of the skin and sclera of the eyes.)

HEMATOCRIT

When whole blood is centrifuged, the red blood cells become packed at the bottom of the tube, leaving the plasma at the top. The ratio of the volume of packed red blood cells to the total blood volume is called the **hematocrit.**

PROCEDURE

1. Prick your finger with a sterile lancet.
2. Obtain a heparinized capillary tube (**heparin** is an *anticoagulant*). Notice that one end of the tube is marked with a red band. Touch the end of the capillary tube opposite the marked end to the drop of blood, allowing blood to enter the tube by capillary action and gravity. The tube does not have to be completely full (half full or more is adequate), and air bubbles are not important (they will disappear during centrifugation).
3. Seal the red-banded (non-blood) end of the capillary tube by gently pushing it upright into clay capillary sealant. Carefully, rotate and remove the tube.
4. Get a partner to spin with. When several partners are ready, place your sealed capillary tube in a numbered slot of the microcapillary centrifuge, with the **plugged end of the capillary tube facing outward** against the rubber gasket. *Have your partner place his/her tube directly across from yours (for balance). Remember your number!* At the end of the centrifugation, determine the hematocrit with a rules:

Hematocrit = height of red cell fraction / height of sample (no units; it's a %)

HEMOGLOBIN CONCENTRATION

Hemoglobin absorbs light in the visible spectrum and hence is a *pigment*. It should therefore be possible to measure the concentration of hemoglobin in a hemolyzed sample of blood by measuring the intensity of its color. This procedure, however, is complicated by the fact that red blood cells contain different types of hemoglobin, and each type absorbs light in a slightly different region of the visible spectrum.

When the oxygen concentration of the blood is high, such as in the capillaries of the lungs, normal **deoxyhemoglobin** combines with oxygen to form the compound **oxyhemoglobin**. When the concentration of oxygen in the blood is low, such as in the capillaries of the tissues, the oxyhemoglobin dissociates to form reduced hemoglobin and oxygen.

Arterial blood is bright red due to the predominance of the oxyhemoglobin pigment, whereas venous blood has the darker hue characteristic of deoxyhemoglobin. It should be emphasized, however, that venous blood, although darker in color, still contains a large amount of oxyhemoglobin; this functions as an oxygen reserve.

MEASUREMENT OF HEMOGLOBIN CONCENTRATION

PROCEDURE:

1. Aliquot 3.0 ml of Drabkin's reagent in a clean cuvette.
2. Clean your finger with alcohol and obtain a drop of blood. You may then either squeeze the drop onto a microscope slide or simply pipette from your finger.
3. Using a P100, pipette 12µl of blood and add the blood to your cuvette. Cover the cuvette with a small piece of Parafilm and invert several times to mix.
4. Incubate the cuvette for 5 minutes at room temperature.
5. Dispose of the pipette tip in the designated biohazzard container.
6. Set the spectrophotometer to 540 nm. Obtain the provided BLANK and STANDARD cuvettes (the class will share these so you don't have to make them yourself).
7. Insert BLANK and press ABS = 0 button to blank the spectrophotometer.
8. Invert the STANDARD cuvette and take the reading.
9. Invert your sample and take the reading.
10. Dispose of your sample in the provided waste beaker and place the cuvette in the designated biohazzard container.
11. Calculate the hemoglobin concentration using Beer's Law. The Hemoglobin standard concentration is 20 g/dL.

2. BLOOD TYPE: ABO and Rh

Materials:

1. Sterile lancets, 70% alcohol
2. Anti-A, anti-B, and anti-Rh sera
3. Slide warmer, glass slides and toothpicks

Red blood cells (RBCs) have characteristic molecules on the surface of their membranes that can be different in different people. These genetically determined membrane molecules are called antigens. The major blood group antigens are the Rh antigen and the antigens of the ABO system.

OBJECTIVES:

1. Explain what is meant by the term *blood type,* and identify the major blood types.
2. Explain how agglutination occurs, and how agglutination tests can be used to determine a person's blood type.
3. Identify the different genotypes that can produce the different blood group phenotypes, and explain how different blood types can be inherited.
4. Explain the dangers of mismatched blood types in blood transfusions.

When blood from one person is mixed with plasma from another person, the red blood cells will sometimes **agglutinate**, or clump together. This agglutination reaction, which is very important in determining the safety of transfusions (agglutinated cells can block small blood vessels), is due to a mismatch of genetically determined blood types.

The Rh Factor

One of the antigens on the surface of red blood cells is the **Rh factor** (named because it was first discovered in rhesus monkeys.). The Rh factor is found on the red blood cell membranes of approximately 85% of the people in the United States. The presence of this antigen on the red blood cells (an **Rh positive** phenotype) is inherited as a dominant trait and is produced by both the *homozygous (RR)* genotype and the *heterozygous (Rr)* genotype. Individuals who have the *homozygous recessive* genotype (rr) do not have this antigen on their red blood cells and are said to have the **Rh negative** phenotype.

PROCEDURE

1. Place one drop of anti-Rh serum on a clean glass slide.
2. Add an equal amount of fingertip blood and mix it with the antiserum (use a toothpick).
3. Place the slide on a slide warmer and rock it back and forth.
4. Examine the slide for agglutination. If no agglutination is observed after a 2-minute period, examine the slide under the low-power objective of the

microscope. The presence of grains of agglutinated red blood cells indicates Rh positive blood.

B. The ABO Antigen System

Each individual inherits two genes, one from each parent, that control the synthesis of red blood cell antigens of the ABO classification. Each gene contains the information for one of three possible phenotypes: antigen A, antigen B, or no antigen (written O). Thus, an individual may have one of four possible phenotypes (A,B,O, or AB) and one of six possible genotypes. This is because unlike many other traits, the heterozygous genotype AB has a phenotype that is different from either of the homozygous genotypes (AA or BB). Since there is no dominance between A and B, individuals with the genotype AB produce red blood cells with *both* the A and B antigens (a condition known as *codominance)* and have **type AB** blood. The most common blood types are type O and type A; the rarest is type AB.

Also, unlike the other immune responses considered, anhtibodies against the A and B antigens are not induced by prior exposure to these blood types. A person with type A blood, for example, has antibodies in the plasma against type B blood even though that person may never have been exposed to this antigen. A transfusion with type B blood into the type A person would be extremely dangerous because the anti-B antibodies in the recipient's plasma would agglutinate the red blood cells in the donor's blood. The outcome would be the same if the donor were type A and the recipient type B:

Antigen on RBC Surface	**Antibody in Plasma**
A (type A)	Anti-B
B (type B)	Anti-A
O (type O)	Anti-A and anti-B
AB (type AB)	No antibody

PROCEDURE

1. Draw a line down the center of a clean glass slide with a marking pencil and label one side **A** and the other side **B.**
2. Place a drop of anti-A serum on the side marked **A** and a drop of anti-B serum on the side marked **B**.
3. Add a drop of blood to each antiserum and mix each with a separate applicator stick.
4. Tilt the slide back and forth and examine for agglutination over a 2-minute period. *Do not heat the slide on the slide warmer.*