Bio 235 Lab: Blood

Part 1. Hematocrit Measurement:

Blood has a number of functions in the body. One of the most obvious is its role in carrying oxygen from the lungs to other parts of an animal’s body. **Hemoglobin** is a protein found in erythrocytes or red blood cells (RBCs). It has a great affinity for oxygen and increases the amount of oxygen that can be carried in the blood. One estimate of the amount of hemoglobin present in blood is the **hematocrit**. Normal hematocrit percentages (%) for humans are 42–52 for men and 37–47 for women. A hematocrit % below these ranges is considered **anemia** (low RBC count). Many things can lead to anemia including blood loss, radiation or chemotherapy treatment, certain cancers, autoimmune disorders, Sickle cell anemia, nutritional problems, and even over-hydration. A high hematocrit %, called **polycythemia**, could indicate dehydration, low oxygen availability (high altitude, respiratory or cardiovascular disease).

**To determine the hematocrit:**
1. Use a sterile lancet to get a blood drop from your finger. Fill a heparinized capillary tube with blood.
2. Seal end of tube will clay sealant.
3. Place capillary tube in numbered position in microcentrifuge. Make sure it is counter-balanced with another tube!
4. When the right number of tubes are in the microcentrifuge the instructor will turn it on for 3 minutes.
5. Recover your tube and calculate the proportion of the red section to the whole column. This proportion is expressed as the **hematocrit percentage**.
6. **Record your hematocrit percentage HERE:** _________________
7. Your instructor might have additional data analysis for you (e.g. Excel spreadsheet)

**Questions:**

What does the red portion of the hematocrit represent? ________________________________________________

What does the clear section of the hematocrit represent? ________________________________________________

Where are the white blood cells found in the hematocrit? ________________________________________________

What possible conditions does a low hematocrit indicate? ________________________________________________

Normal ranges for humans are 42-52 for men and 37-47 for women. Why is there this difference? ________________________________________________

Besides carrying oxygen bound to hemoglobin, blood cells also function in immunity by destroying diseased cells and foreign bodies (viruses, bacteria, fungi). There are five different types of white blood cells (WBCs) or leukocytes that play differing roles in an animal’s defense. Granulocytes are WBC with granules within their cell cytoplasm. Granulocytes include neutrophils, eosinophils, and basophils. Neutrophils are the most abundant WBC and function in quick (innate) immune responses and are usually the first WBCs at the scene of an inflammation. Eosinophils are WBCs that are seen with chronic inflammation and infection, allergies, and parasites. Eosinophil granules often stain bright pink/red/orange with the Wright’s stain. Basophils are the most unusual granulocytes and have darkly staining granules. Basophils play a role in mediating inflammatory reactions involving histamine. Agranulocytes are WBCs that lack granules within the cytoplasm and they include lymphocytes and monocytes. Lymphocytes (B cells, specifically) are involved in humoral immunity in which they secrete antibodies that bind to antigens on the surface of invading cells and flag them for destruction. Monocytes are agranular WBC that migrate quickly to the site of inflammation & infection to destroy foreign cells and can migrate into the tissues (as macrophages) to continue to defend the body.

Below is a listing of normal ranges for WBCs in human blood:

<table>
<thead>
<tr>
<th></th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUMAN %</td>
<td>55 – 75%</td>
<td>1-3%</td>
<td>&lt; 1%</td>
<td>25-35%</td>
<td>4-6%</td>
</tr>
</tbody>
</table>

*NOTE – Different sources might cite slightly differing percentages.*

To examine white blood cells.

1. **Your instructor might have you examine already-prepared blood smears. If so, go directly to step 4!** If your instructor wants you to make your own blood smear slide follow steps 2 & 3.
2. After the blood has dried cover the surface with Wright’s stain for two minutes. When adding the stain, count the number of drops. After two minutes add the same number of drops of DI water. Allow the diluted stain to cover the smear for four minutes.
3. Gently wash away stain with water, blot underside of the slide dry with a paper towel, then set slide aside briefly to dry.
4. Look at the blood cells using a microscope. *[If you are unfamiliar with using a microscope please see your instructor first!] Start first at a lower magnification and adjust the course focus, then when the cells are visible you can switch to a higher magnification and use the fine focus to bring the cells into view. Scan your smear in a regular manner (up then over and then down and over) and count the numbers of each type of each white blood cell. Stop when you reach ~100 white blood cells.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>NEUTROPHIL</th>
<th>EOSINOPHIL</th>
<th>BASOPHIL</th>
<th>LYMPHOCYTE</th>
<th>MONOCYTE</th>
</tr>
</thead>
<tbody>
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<td>HUMAN %</td>
<td>55 – 75%</td>
<td>1-3%</td>
<td>&lt; 1%</td>
<td>25-35%</td>
<td>4-6%</td>
</tr>
<tr>
<td>YOUR SAMPLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Questions:

How does your sample compare to the normal? ______________________________________

Part 3. Blood typing

B lymphocytes produce antibodies (or immunoglobulins) that bind to antigens (molecules on the surface of cells) and cause a cascade effect in the immune system that destroys the cells bound by antibodies. The surface of red blood cells contains a group of antigens that distinguish a blood type within the ABO blood type system of humans.

1. Blood type A has RBCs with A antigens (agglutinins) and anti-B antibodies. Anti-B antibodies in Type A blood will cross react against the B antigens in Type B blood; thus, Type A people can receive blood only from Type A or Type O people.

2. Blood type B has RBCs with B antigens and anti-A antibodies. Anti-A antibodies in Type B blood will cross react against the A antigens in Type A blood; thus, type B people can receive blood only from Type B or Type O people.

3. Blood type AB has RBCs with both A and B antigens but lacks any antibodies. Without these antibodies Type AB blood will not cross react with either A or B antigens. Thus, Type AB people can receive blood from Type A, Type B, and Type O people. This is why Type AB people are called “universal recipients”.

4. Blood type O has RBCs with no antigens. Thus, there are no antigens to cross react with either anti-A or anti-B antibodies. Type O people can donate blood to either Type A, Type B, or Type AB people and they are called the “universal donor”. However, Type O people can only receive blood from other Type O people. Fortunately, Type O blood is one of the most common blood types (Table 2).

Rh factor (Rhesus factor) is one of the human blood group systems outside of ABO. The Rh blood group system consists many antigen groups; however antigen D is the most important in determining potential cross-reactivity in blood transfusions or hemolytic anemia in newborns. If someone is Rh- (neg) they lack the D antigen and if they are Rh + (pos) they have the D antigen. For example, someone who is A- is Type A lacking the Rh D antigen. Likewise, someone who is A+ is Type A having the Rh D antigen.

What antibodies does Type O blood have? __________________________

What antibodies does Type AB blood have? __________________________

What antigens does Type A blood have? __________________________

How to Determine Your Blood Type:

1. Place two drops of blood into each of two wells of the blood typing card where indicated.

2. Add anti-A to one drop and anti-B to the other. [Be careful not to touch the tip of the bottle to your blood sample because that would contaminate the bottle!]

3. Observe any agglutination in the samples and determine your blood type. ______________________
You and your instructor will summarize the blood types in the class according to Table 2 below. How do the percentage of blood types reported for the class compare with those reported in Table 3 below?

![Table 2](image)

![Table 3](image)

*Source: Stanford School of Medicine Blood Center (http://bloodcenter.stanford.edu/about_blood.htm)*