Laboratory 12
Blood Cells

Objectives:
• Identify microscopically each of the following: erythrocytes (red blood cells or RBCs), the five types of leukocytes (white blood cells or WBCs), and thrombocytes (platelets).
• Compare and contrast the morphological features of erythrocytes and the five types of leukocytes.
• State the normal ranges for erythrocyte counts and hematocrit (both male and female), total leukocyte count, and platelet
• List the five types of leukocytes in order of their relative prevalence in normal blood and classify each type as granulocyte or agranulocyte.
• Explain how platelets differ structurally from the other formed elements of the blood.

The human body contains 4-6 L of blood. Blood is composed of a liquid extracellular matrix called plasma and formed elements including erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). Leukocytes can further be broken down into granulocytes, cells that contain extensive cytoplasmic granules, and agranulocytes, which lack the staining granules in the cytosol. Different types of leukocytes can be differentiated from each other based on overall shape, nuclear shape, and staining of the cytoplasmic granules.

A number of diagnostic measures focus on the relative abundance of formed elements in the blood including the overall number/volume of erythrocytes as measured by a hematocrit, the overall number of leukocytes, and the relative abundance specific types of leukocytes.

This lab will first, histologically examine the different types of formed elements seen in a human blood smear. We will then use images of blood smears to perform differential white blood cell counts of case study purposes. Finally, we use a hematocrit to determine the amount of erythrocytes in a blood sample.

1. Blood Cell Histology
Erythrocytes are also called red blood cells because, even unstained, they appear red in color. The color is due to the presence of a protein called hemoglobin which reversibly binds to oxygen. Most oxygen in the blood is carried in red blood cells bound to hemoglobin. Red blood cells are small, anucleate cells that appear as biconcave discs (Figure 1).

Thrombocytes, or platelets, are a type of formed element found in the blood. Platelets are small pieces of cells that lack a nucleus or most organelles (Figure 1). They contain secretory vesicles that release components that stimulate aggregation of the platelets, an important component in blood clotting.
Leukocytes are the larger, nucleated cells seen in the blood smear. The cells will be differentiated from each other based on overall cell shape, the shape of the nucleus, and presence and staining of cytoplasmic granules. These cells function in the body's immune response. Images and specific description of the cells are in Figure 2.

Activity:
Use the prepared normal blood smear slides to view blood cells microscopically.

These are optimally viewed at 1000X. The 100X objectives are oil immersion objectives. It is crucial that you very carefully follow the correct steps for using an oil immersion objective. You first find the cells on the 4X objective, then find them on the 10X and focus, then find them on the 40X and focus. Now turn the objective so that you are half way between the 40X and the 100X objectives. Now place 1 or 2 drops of immersion oil onto the slide at exactly the spot that the light is shining through. Now carefully lower your 1000X objective into the oil and fine focus to optimally view the cells. DO NOT USE THE COARSE FOCUS ON THE 100X objective. If you cannot find the cells you will need to very carefully move the objective to half way between the 40X and 100X objectives and clean all of the oil off of the slide with a KimWipe and lens cleaner before going back to the 40X objective. Make sure that you do not get any oil on these 40X objectives as they are almost impossible to get the oil off.

Do all of these drawings as close to the size that you see them in your field of vision as possible. Do not artificially make them bigger than they actually appear to you at 1000X.

Drawings:
- Segmented Neutrophil also include some red blood cells and platelets for size comparison.
- Lymphocyte also include some red blood cells and platelets for size comparison.
- Monocyte also include some red blood cells and platelets for size comparison.
- Eosinophil also include some red blood cells and platelets for size comparison.
- Basophil also include some red blood cells and platelets for size comparison.

Make sure that you properly label your drawings.

<table>
<thead>
<tr>
<th>Cell Picture</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Seg neutrophils" /></td>
<td>Seg neutrophils are mature neutrophils. Neutrophils are phagocytic cells that are important in the body's response to invading bacteria.</td>
</tr>
<tr>
<td><img src="image" alt="Band neutrophils" /></td>
<td>Band neutrophils are new, immature neutrophils. They have band-shaped nuclei that will segment later.</td>
</tr>
</tbody>
</table>
Lymphocytes have large round nuclei and clear cytoplasm. These cells either mature in the bone marrow (B-cells) and secrete antibodies or mature in the thymus (T-cells) and are involved in the adaptive cellular immune responses.

Atypical lymphocytes have jagged edges. Look at the membrane, not the nucleus!

Monocytes have light blue curved nuclei and clear cytoplasm. These cells are phagocytic and become macrophages when they leave the blood and enter the tissue.

Eosinophils have bright red granules in their cytoplasm. Some of the granules are made of worm-fighting proteins.

Basophils have dark purple granules made of histamine in their cytoplasm. Histamine causes allergy, asthma and anaphylactic shock. Basophils become mast cells when they leave the blood.

Figure 2. Types of leukocytes seen in a human blood smear.

2. Differential Blood Cell Count:

Several blood parameters are routinely used to identify potential illnesses.

A complete blood count (CBC) is one of the most common blood tests. A CBC includes the hematocrit, the hemoglobin content, the total white blood cell count and the platelet count. These tests are used to evaluate the number and types of cells present in the blood and compare them to normal levels. It is important to draw blood into a tube with an anticoagulant to prevent its clotting if a CBC is to be done.

A hematocrit is done to determine the percentage of erythrocytes in the blood. A low hematocrit might indicate some type of anemia and a high hematocrit might indicate dehydration. For males a normal hematocrit is 47 with a range of 40-54 and for females the average is 42 with a range of 37-47.
The total leukocyte count can be taken to indicate whether the immune system is in balance. If the total leukocyte count is off, a differential white blood cell count can indicate which type of white blood cell is present in unusual numbers as seen below. The normal range of total leukocyte count is 4500-11,000/mm³.

A platelet count can indicate blood clotting issues. The normal range is 50–400 x 10⁹ per liter.

**Activity**
- Do this activity as a table team.
- Each person needs a patient folder for one of the five patients. If there are four people at your table, do any four of the five patients. If there are five people at your table, do all five patients.
- First, read about your patients:
  - One of them is normal.
  - One has AIDS. Her lymphocyte count is low because HIV destroys Helper T lymphocytes. Her other WBCs percentages *seem* to be high, but that is only an illusion. Here is an example. Pretend you have a fruit basked with five oranges and five apples. Each fruit is 50% of the total. Now take four apples out of the basket. The *percentage* of oranges in the basket increases to 83% even though their number has not changed. In the same way, decreasing the number of lymphocytes increases the *percentage* of neutrophils even though their number has not changed. That is why the AIDS patient’s elevated neutrophil percentage does not necessarily mean she has a bacterial infection too.
  - One has acute bacterial infection. His neutrophil count is high because brand new neutrophils are rushing into his blood to fight bacteria. New neutrophils are called bands. Mature neutrophils are called segs. He has more of those too.
  - One has viral mononucleosis. Her lymphocyte count is high because more lymphocytes are rushing into her blood to fight viruses. Her lymphocytes look atypical with jagged edges. Her spleen is swollen. She is a high school student.
  - One has an acute parasite infection with a worm called Trichinella because he ate undercooked bear meat. His eosinophil count is high because eosinophils are rushing into his blood to fight worms.
- Start in the upper left corner of the first page in the folder. Identify the white blood cell in the first square and put a mark by its corresponding picture on page 143. Move across to the next square and then the next square and so forth. Some squares have two WBCs. In that case, identify and record both. Continue on the second page. Your total count should reach 100 because there are 50 WBCs on each page.

**Warning!** Do not go through and count all the neutrophils, then go through and count all the lymphocytes, then go through and count all the monocytes, etc. Go through just once to minimize errors.
<table>
<thead>
<tr>
<th>Type of Cell</th>
<th>Use for Counting</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seg Neutrophil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band Neutrophil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of Leukocyte</th>
<th>Granulocyte/Agranulocyte</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>Granulocyte</td>
<td>50-70%</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>Agranulocyte</td>
<td>20-30%</td>
</tr>
<tr>
<td>Monocyte</td>
<td>Agranulocyte</td>
<td>2-8%</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>Granulocyte</td>
<td>2-4%</td>
</tr>
<tr>
<td>Basophil</td>
<td>Granulocyte</td>
<td>less than 1%</td>
</tr>
</tbody>
</table>

### 3. Hematocrit

- Obtain a heparinized capillary tube and a capillary tube sealer.
- Immerse the red-marked end of the capillary tube in the blood sample and fill it three-quarters full. Plug the blood-containing end by pressing it into the capillary tube sealer.
- Place the prepared tube in the radial grooves of the microhematocrit centrifuge with the sealed ends against the rubber gasket at the centrifuge periphery. Make sure the centrifuge is balanced and secure the centrifuge cover. Turn the centrifuge on and set the timer for 4 minutes.
- When the centrifuge has come to a complete stop, remove your tube and align it with the microhematocrit reader card. Determine the percentage of RBC’s, WBC’s and plasma. The RBC’s are the bottom layer, the plasma is the top layer and the WBC’s are the layer between the two.
Attributions:

Objectives taken from the HAPS guidelines.


Differential activity used with permission from a really great biology instructor from a community college in Oregon. Sadly his name isn’t on the materials and I can't find it anywhere.
Activity 1: Histology Pages at back.

Activity 2: Results

1. What is your patient’s number? It’s on the folder. __________

2. Compare your counts with normal percentages given below and diagnose your patient’s condition.
   Your counts are percentages because you counted 100 WBCs.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Your Patient</th>
<th>Is your patient normal?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seg neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-65%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Consult with your table team and fill in these blanks. Leave one of the blanks empty if you are a team of four.

   Patient 1’s diagnosis: ____________________________________________
   Patient 2’s diagnosis: ____________________________________________
   Patient 3’s diagnosis: ____________________________________________
   Patient 4’s diagnosis: ____________________________________________
   Patient 5’s diagnosis: ____________________________________________

4. Consult with your team to answer these questions:

   What would be a good question to ask your patient, other than “Did you eat bear meat?”, if your patient has a high percentage of eosinophils? Hint: Where are parasites more common in the world?

Lymphocyte counts usually increase when patients have viral infections. So why are
lymphocytes counts low in HIV patients? HIV is a virus.

Which one of these patients most likely got infected during surgery? Why?

What concerns should you raise with your mono patient? Remember, she is a high school student.

Activity 3 Results:

%RBC __________ %WBC ____________ %plasma __________

1. Define hematocrit:

2. If you had a high hematocrit, would you expect your hemoglobin determination to be high or low?
   __________
   Why?

3. Micahela is an athlete who has been training for several years only in Florida. She had an annual physical and the doctor read her hematocrit as 59%. What could this reading potentially tell the doctor?