

## Exercise 2: Blood



Figure 2.1 This image of erythrocytes (red blood cells) was taken with a scanning electron micrograph and its color was added digitally. What unique features of RBCs can you see in this micrograph?

### Exercise 2 Learning Goals

After completing this lab you should be able to:

- Describe the functions of blood.
- Describe the cellular and extracellular components of blood, including plasma and the formed elements.
- Identify the formed elements of blood in a histological section.
- Identify blood types and explain the physiological basis of blood type.
- Be able to perform a basic blood typing test.
- Perform a hematocrit and explain the clinical relevance of the hematocrit.
- Perform a whole blood smear with fixation and staining.
- Use a microscope to view a fixed whole blood smear and identify formed elements.
- Describe different diagnostic blood tests and blood diseases.

# Pre-Laboratory Exercise 2

## Pre-Lab Activity 2.1

In the table below, describe the functions of blood within the body.

Web Resources:

<https://cnx.org/contents/FPtK1zmf@15.2:1UrEdFyf@11/18-1-An-Overview-of-Blood>

[https://www.histology.leeds.ac.uk/tissue\\_types/index.php](https://www.histology.leeds.ac.uk/tissue_types/index.php)

Function	Description
Distribution	
Regulation	
Protection	
Transportation	

## Pre-Lab Activity 2.2 Blood Components

In the chart below describe the visual appearance and function of each of the blood components.

	Function	Description of Appearance	Sketch
<b>Extracellular Matrix</b>			
<i>Plasma</i>	Protein-rich fluid that makes up 55% of blood; 91-92% water, 7-8% proteins, 1-2% other solutes including electrolytes, nonprotein nitrogenous substances (urea, creatine), blood gases (oxygen, carbon dioxide, nitrogen), and regulatory substances (hormones, enzymes)		
<b>Formed Elements</b>			
<i>Erythrocytes (Red Blood Cells)</i>	Specialized for the transport of oxygen and carbon dioxide	Bi-concave discs with no nucleus; possess surface antigens (ex. A, B, O)	
<i>Leukocytes (White Blood Cells)</i>			
<i>Neutrophils</i>	The most common of the leukocytes	Cytoplasm does not stain dark; nucleus is composed of 3-5 lobes	
<i>Eosinophils</i>		Cytoplasm stains red; nucleus composed of two lobes (bilobed)	
<i>Basophils</i>		Cytoplasm stains dark purple; nucleus is S-shaped	

<i>Lymphocytes</i>		Large spherical nucleus with slender region of cytoplasm that stains blue	
<i>Monocytes</i>		Cytoplasm stains blue and U-shaped nuclues	
<b><i>Thrombocytes (Platelets)</i></b>		Small	

## Pre-Lab Activity 2.3 Identify Formed Elements

In the image below circle an example of a red blood cell, white blood cell and platelet.

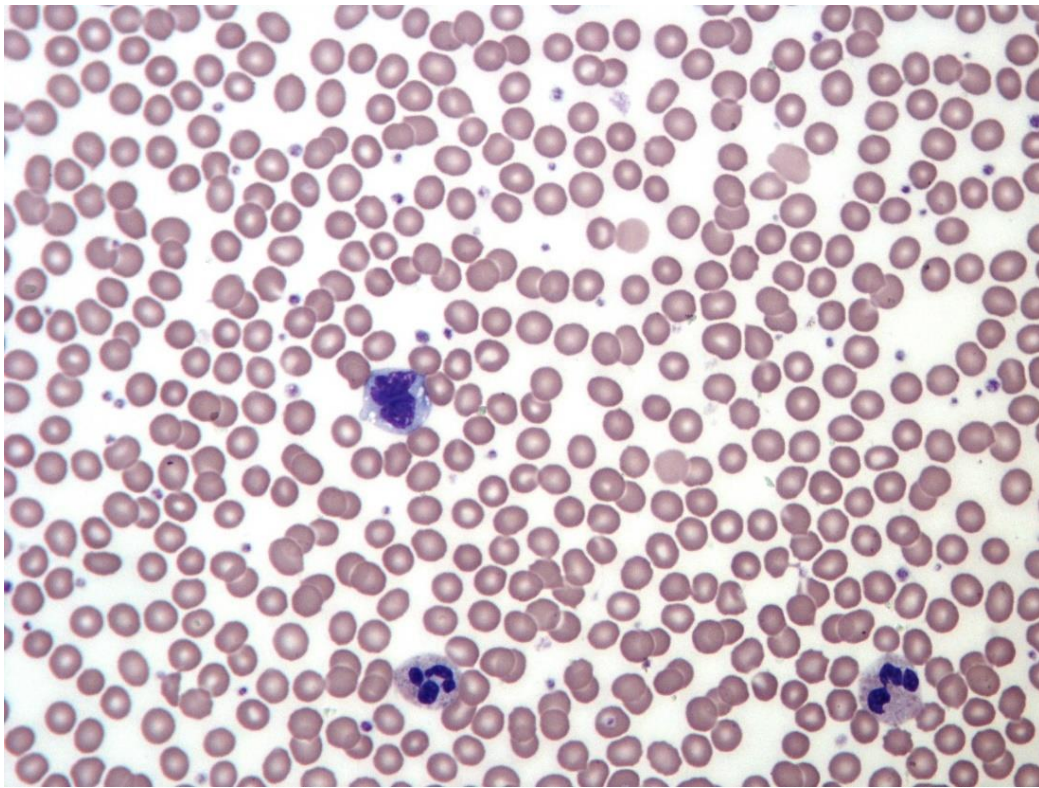


Figure 2.2 The histological image above is of a normal human adult blood smear with a Giemsa staining.

[https://commons.wikimedia.org/w/index.php?search=blood+smear&title=Special:Search&go=Go&ns0=1&ns6=1&ns12=1&ns14=1&ns100=1&ns106=1&searchToken=81h7jb09aecul15jp0kgqd1ch#%2Fmedia%2FFile%3ANormal\\_Adult\\_Blood\\_Smear.JPG](https://commons.wikimedia.org/w/index.php?search=blood+smear&title=Special:Search&go=Go&ns0=1&ns6=1&ns12=1&ns14=1&ns100=1&ns106=1&searchToken=81h7jb09aecul15jp0kgqd1ch#%2Fmedia%2FFile%3ANormal_Adult_Blood_Smear.JPG)

## Pre-Lab Activity 2.4 Blood Types

Blood is a liquid connective tissue that is important in gas exchange and nutrient distribution to most body cells. In the watery extracellular matrix of blood, known as blood plasma, there are three formed elements: 1. erythrocytes (red blood cells), 2. leukocytes (white blood cells) and 3. cell fragments known as platelets. Red blood cells are most abundant and have a genetically unique assortment of glycoproteins and glycolipids embedded in their plasma membranes. These glycoproteins and glycolipids serve as antigens and occur in predictable combinations. The presence or absence of the characteristic antigens determine which blood group and blood type an individual has. There are 24 blood groups and over 100 antigens found on the surface of red blood cells. For the purposes of this lab we will only discuss the ABO and Rh blood groups. The ABO blood group is based on whether individuals possess glycolipid antigens known as A and B. Individuals with red blood cells that have only A glycolipids would have type A blood, those with B glycolipids would have type B blood and some individuals are co-dominant for type A and B glycolipids which means their red blood cells display both glycolipids as type AB blood. An individual that lacks A or B antigens on red blood cells has type O blood (see figure 2.3). The Rh blood group determines whether your blood type is positive or negative and is named because the antigen was first discovered in the *Rhesus* monkey. Individuals that are positive for Rh antigens have these antigens displayed on the plasma membrane of their red blood cells. Individuals that are negative for Rh antigens do not have these antigens displayed on their red blood cells. The combination of these antigens displayed on your red blood cells determines your blood type which may be A<sup>+</sup>, A<sup>-</sup>, B<sup>+</sup>, B<sup>-</sup>, AB<sup>+</sup>, AB<sup>-</sup>, O<sup>+</sup> or O<sup>-</sup>.

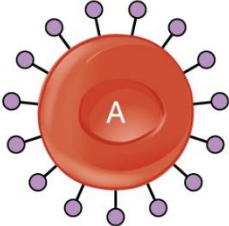
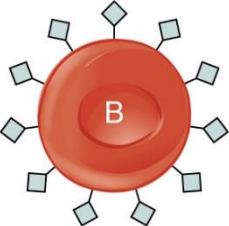
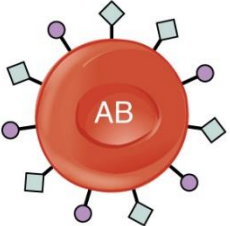
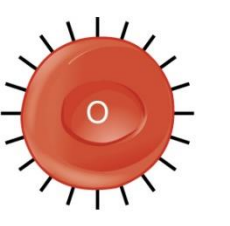

































Blood Type				
	A	B	AB	O
Red Blood Cell Type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red blood Cell	 A antigen	 B antigen	 A and B antigens	None
Blood Types Compatible in an Emergency	A, O	B, O	A, B, AB, O (AB <sup>+</sup> is the universal recipient)	O (O is the universal donor)

Figure 2.3 Table illustrating ABO blood types and compatible blood types for transfusions.

[https://commons.wikimedia.org/w/index.php?search=abo+blood+groups&title=Special%3ASearch&go=Go&ns0=1&ns6=1&ns12=1&ns14=1&ns100=1&ns106=1#/media/File:1913\\_ABO\\_Blood\\_Groups.jpg](https://commons.wikimedia.org/w/index.php?search=abo+blood+groups&title=Special%3ASearch&go=Go&ns0=1&ns6=1&ns12=1&ns14=1&ns100=1&ns106=1#/media/File:1913_ABO_Blood_Groups.jpg)



To better understand blood types and their importance study the blood type compatibility chart below and answer the following questions.

		DONOR BLOOD TYPES							
		O-	O+	B-	B+	A-	A+	AB-	AB+
RECIPIENT BLOOD TYPES	AB+								
	AB-								
	A+								
	A-								
	B+								
	B-								
	O+								
	O-								

1. If a person that was A<sup>+</sup> needed a blood transfusion, would they be able to receive AB<sup>+</sup> donor blood? Explain.
2. Would a person with AB<sup>+</sup> blood be able to receive a transfusion from an O<sup>+</sup> donor? Explain.
3. Would a person with O<sup>-</sup> blood be able to receive a transfusion from a B<sup>-</sup> donor? Explain.
4. Which blood type can be used universally for transfusions?
5. Which recipient blood type can receive any donor blood type?
- 6.

**Pre-Lab Activity 2.5 Identify the White Blood Cell Type**

White blood cells are a major formed element of blood and important in immune activity. White bloods cells are also known as leukocytes and are significantly larger than both red blood cells and platelets, but numbers are much fewer. White blood cells have uniquely shaped nuclei and are characterized as granular or a granular depending on whether their granules are easily viewed when stained.

Using figure below match the image to the type of leukocyte: Basophil, Eosinophil, Monocyte, Lymphocyte or Neutrophil. Then complete the description underneath highlighting the unique features of the particular white blood cell.



<b>Cell Type:</b>	<b>Cell Type:</b>	<b>Cell Type:</b>	<b>Cell Type:</b>	<b>Cell Type:</b>
<b>Description:</b>	<b>Description:</b>	<b>Description:</b>	<b>Description:</b>	<b>Description:</b>

Image from OpenStax



## Lab Exercise 2: Blood

### Important Safety Note:

**At no time will any student handle blood or other biohazard material that is not their own.**

- Before beginning any of the lab procedures, be sure to read through the entire protocol first. Gather all of the materials you will need for each test, lay them out at your work station, and ensure you have not missed any steps in the procedure or materials before you start.
- You will want a couple of paper towels underneath where you will be working in case of spills in addition to some extra towels for blotting your finger. Always wear your protective goggles and gloves while you or anyone else in the lab are handling any chemical or biohazard materials. Do not touch or handle anyone else's biohazard materials.
- All materials that have come into contact with human blood are to be disposed of in the red biohazard bags on each benchtop. This includes used alcohol wipes, lancets, gloves, microcapillary tubes and slides/coverslips.
- Always wash your hands with soap and water before and after wearing gloves.
- Before obtaining your own blood sample, warm up your hands and fingers by massaging or rubbing them together. It will be difficult to obtain even a tiny blood sample if your hands are cold. Keeping your hands below the level of your heart, focus on the index finger of your non-dominant hand since this is where you will likely get the best sample for your tests. Firmly rub your index finger working from the palm down to the tip of the finger. You can usually see the fingertip pinken and feel it warm up as blood flow increases to the area. When your hand feels sufficiently warm, place a glove on the hand you WILL NOT be finger-sticking and proceed through the test protocol.

## Activity 2.1: Hematocrit

Supplies needed:

- ☐ Gloves
- ☐ Alcohol wipe
- ☐ Safety lancet
- ☐ Capillary tube
- ☐ Capillary tube sealant
- ☐ Ruler

1. Put on safety goggles, wash your hands, and put on your disposable gloves.
2. When you are ready to obtain a blood sample from yourself, remove glove from one hand. Warm your hand up and keep it below the level of your heart when obtaining a sample. Use an alcohol wipe to thoroughly clean the tip of your index finger on the hand where you removed your glove. Dispose of the alcohol wipe in the biohazards container.
3. Remove the cap from your safety lancet and set it on the workspace in front of you. Wipe the pad of the fingertip you plan to use with an alcohol prep pad and set it aside for disposal. Once you have wiped a finger, allow it to air dry briefly and do not touch anything with the now-prepped area. Press the safety lancet to the prepped area and apply pressure until a distinct “click” is heard. Immediately dispose of the lancet in the red biohazard bag.
4. Hold the opening of your capillary tube at an angle to the forming blood droplet. Fill the capillary tube half to 2/3rds full of blood, squeezing the finger if necessary to extract more blood. More blood is better than not enough for this test.
5. Once you have collected enough blood in your tube, hold your gloved finger over the blood collection end of the tube and press the dry end into the sealant clay. Be careful as the tube is glass and fragile.
6. At this time it is advised to use the same finger-stick for other tests. Set your microcapillary tube aside with the open end propped up.

7. Apply a **large** drop of blood to each of the three spaces on your blood typing card and set aside for Activity 2.
8. Apply a large drop of blood to a sheet of ChemTek paper, leaving a complete saturation drop, and set aside to dry for Activity 4.
9. Now that your capillary tube is sealed, give it to your instructor to place into the centrifuge. The tube should be placed in a groove with the sealed end towards the outside edge of the centrifuge and the open end facing the center. Notate the number associated with your tube placement below.

**Centrifuge slot number:** \_\_\_\_\_

10. Your instructor will run the centrifuge for 90-120 seconds. Promptly return for your sample to allow additional groups to be run.
11. Use a hematocrit reader or a basic metric ruler to interpret the hematocrit and complete the exercise below. You may lay the tube on a piece of paper and mark the divisions between blood components if you want to return to take measurements later in lab. However, be sure your lines are correct before disposing of your tube in the biohazard bag.

### **Exercise 2.1: Determining the Hematocrit**

In the space provided below, mark the separation between the erythrocytes (at the bottom of the tube), the buffy coat (in the middle), and the plasma (the clear liquid at the top).

Length of the entire blood column (mm): \_\_\_\_\_

Length of the erythrocytes (mm): \_\_\_\_\_

Length of the buffy coat (mm): \_\_\_\_\_

Length of the plasma (mm): \_\_\_\_\_

Calculate the percentage of erythrocytes in the blood. This calculation is known as the hematocrit.

Hematocrit= Length of erythrocytes (mm)/ whole length of blood column (mm) x 100%

\_\_\_\_\_ = \_\_\_\_\_ / \_\_\_\_\_ x 100%

### Activity 2.3: Blood Typing

Supplies needed:

- ☐ Gloves
- ☐ Alcohol wipe
- ☐ Safety lancet
- ☐ Blood typing card
- ☐ Anti-A serum
- ☐ Anti-B serum
- ☐ Anti-D (Rh) serum
- ☐ Stir sticks

1. Using the finger that you pricked in the previous activity, or repeating the same finger-stick procedure from Hematocrit, apply a large drop of blood within each of the three designated sections on the blood typing card.
2. Add a drop of Anti-A serum to the blood sample in the Anti-A section. Use a clean stick to gently stir the serum into the sample. Discard stick and do not use again. Repeat this process twice more- with the Anti-B and Anti-D serums, respectively.
3. Allow your sample time to fully process, but do not allow the samples to dry out. Add more anti serum if needed.

### Exercise 2.2: Determine your Blood Type

Analyze the typing card and determine your blood type based on the reactions that did or did not occur. Check the appropriate box based on what you see. You may also photograph your typing card if you wish.

Anti-Serum A:      Reaction \_\_\_\_\_      No Reaction \_\_\_\_\_

Anti-Serum B:      Reaction \_\_\_\_\_      No Reaction \_\_\_\_\_

Anti-Serum D:      Reaction \_\_\_\_\_      No Reaction \_\_\_\_\_

Blood Type: \_\_\_\_\_

### Activity 2.4: Hemoglobin

Supplies needed:

- ☐ ChemTek Paper
- ☐ Hemoglobin analysis chart

1. Follow the same finger-prick protocol as you did in Hematocrit to obtain a blood sample if you did not already. When you have a droplet of blood, apply it to the ChemTek paper and set aside to dry.
2. Once the blood sample has dried, it may be analyzed using the hemoglobin chart provided.

### Exercise 2.2: Hemoglobin Saturation

1. Use the analysis chart to compare colors and determine the relative oxygen saturation percentage of your blood. Be sure that you are comparing the darkest site of blood saturation on the paper and not a lighter area of potential smear. Record your O<sub>2</sub> saturation below.

S<sub>PO2</sub>= \_\_\_\_\_

## Activity 2.5: Histological Examination of Blood

Supplies needed:

- ☐ Nitrile gloves
- ☐ Alcohol prep wipe
- ☐ Safety lancet
- ☐ Microcapillary tube
- ☐ Clean glass slides (x2)
- ☐ Wax pencil

After fixation & staining:

- ☐ Compound microscope
- ☐ Coverslip

### Activity 2.5.1 Whole Blood Smear

1. Begin by obtaining all the supplies needed and laying them out at your workstation. Be sure you work on top of several paper towels.
2. Mark the end of one slide with your initials. This will be called Slide 1 and it will be the one you perform the blood smear **ON**. The second slide (Slide 2) will only be used as a tool to help you smear the blood sample across Slide 1.
3. Remove the cap from your safety lancet and set it on the workspace in front of you. Wipe the pad of the fingertip you plan to use with an alcohol prep pad and set it aside for disposal. Once you have wiped a finger, allow it to air dry briefly and do not touch anything with the now-prepped area. Press the safety lancet to the prepped area and apply pressure until a distinct “click” is heard. Immediately dispose of the lancet in the red biohazard bag.
4. You should now have a rapidly forming blood droplet at the site of your finger-stick. If very little blood is visible, gently rub the finger proximally to distally with your other hand to increase blood flow. Using the microcapillary tube, collect the blood droplet using the same procedure from the Hematocrit protocol. Only a small amount of blood is needed. Blot or apply pressure to stop any bleeding from your finger, then re-glove.
5. Gently apply a single, small dot of blood using the microcapillary tube approximately 1-2 cm from your initials on Slide 1. Dispose of the tube in a biohazard bag.



6. Remember, only ever handle microscope slides by touching their outer edges.

Hold the short end of Slide 2 against Slide 1 on the other side of the blood droplet from your initials at a 30-45° angle over the sample. Note that the edge of Slide 2 should be closer to the middle of Slide 1 than the blood sample or your initials are at this point.

7. Continue holding Slide 2 at a 30-45° angle against Slide 1 and “back it up” to the blood sample slowly. When the edge of Slide 2 makes contact with the blood, capillary action will pull the sample out in a line along the edge of Slide 2 where it is touching Slide 1. Keeping the edge of Slide 2 in contact with Slide 1 at all times, quickly “push” Slide 2 across Slide 1 away from your initials toward the other short edge. This action helps spread the cells out into a thin layer for microscopic analysis. Dispose of Slide 2 in biohazard after you have successfully completed a smear on Slide 1.
8. Allow the smear on Slide 1 to completely air dry. Do not proceed to fixation and staining until all moisture has evaporated. Beginning with just a small blood drop and performing a long smear will ultimately yield a better slide in the end. It will dry much more quickly and provide better views under the microscope than slides with too much blood or not enough “smear”.

### **Activity 2.5.2 Fixation & Staining**

1. Once fully dried, take your blood smear slide to the staining station next to the sink.
2. Clip a clothespin onto the short edge of the slide where your initials are written. Hold the slide by the clothespin only just above the countertop to test that the slide is being held securely before proceeding.
3. For each of the 3 dipping stations, hold the slide using the clothes pin and only dip far enough to cover the blood smear. To reduce dripping and mess, keep the clothespin from going into each solution.
4. Dip the slide completely in and out of Solution 1, for 3-5 times in 5 seconds. Gently tap the slide against the inside of the container above the solution to remove any excess before moving to Solution 2.
5. Repeat the slide dipping 3-5 times in 5 seconds for Solution 2. Follow the same procedure to remove excess solution.

6. For Solution 3, perform the same dipping action 3-5 times in 5 seconds. After following the same procedure to remove excess solution, remove the clothespin and turn the sink on to a low flow. **DO NOT** place your slide under the running tap water!
7. Using one of the angled spout bottles of distilled water, hold your slide over the sink and use distilled water to finish removing all remaining excess stain from the slide. Prop your slide in the drying tray along its long edge. Make sure you can still read your initials and identify your slide if there are others beside it. Leave slide to air dry completely.
8. Clean up your workstation and dispose of all used materials properly. Wipe down your table and return clean, unused materials to their original places. Set up a compound microscope at your station and make sure it is in good working order.

### Activity 2.5.3 Histological Examination of Blood

1. Once your fixed and stained slide is completely dry, bring it to your microscope for viewing.
2. Beginning with the 4x power objective, adjust your course then fine focus. Look over your slide and determine where cells are spread thinly, usually towards the end of your smear, this will allow you to visualize individual cells.
3. Increase the objective power to 10x and move the microscope stage as needed to view other areas. Be on the lookout for leukocytes. Use the fine focus to improve clarity.
4. Before moving to the 40x power, place a clean coverslip (touching only the edges!) over the area of your slide you have chosen to focus on. You may need to swivel the 10x objective out of the way to remove and then replace your slide in the stage clips.
5. Once your coverslip is in place, you may move to the 40x objective. Remember to only use fine focus at this power and SLOWLY turn the stage control knobs to look at other areas. Begin to document the different cells present in the sample.

### Exercise 2.3: Identifying Cellular Components of Blood

Using the 40x objective scan your blood smear and see how many different components of whole blood you can identify. Use the pre-lab exercise, your textbook, and/or a reputable online source to help you identify the specific blood cell types in the sample.

Take some photographs with your smartphone camera through one of the eyepieces when you find unique cell types. Work together with your lab partner to identify everything you find and complete the table on the following page.

Sketch of Unique Cell at 40x Objective	Description of Appearance	Presumed Cell Type