

Lab 3 (part 2)

Blood

Study Tip:

Your main job is to be able to recognize the different types of cells on the Blood Smear slide (Slide 2-3-1), and be able to interpret a Blood Type Card. Once you got that down, study the details of each cell noted here. I will explain in the demo what details of the cells listed below are “*more testable*” than others, and which are mostly just “*cool facts*” I typed for fun.

Slide 2-3-1 Blood*:

*This is a prepared slide of “Whole Blood” prepared with Wright’s Stain. You can also choose to look at your own blood!

— All the formed elements of the blood are formed (and recycled) in the red bone marrow, the liver or the spleen.

□ **Erythrocyte** (Red Blood Cell)

- Mature erythrocytes (RBCs) are **anucleate biconcave disks**.
- the cytoskeletal protein **Spectrin** that lines the intracellular side of the plasma membrane give the cell its flexible shape.
- RBCs have a diameter of about **7.5 μm** .
- each RBC contains about 270 million **hemoglobin** molecules!
- **Oxygen** binds to the **Iron (Fe)** on the Hemoglobin.
- RBCs circulate for about **100 to 120 days** before they break apart and die.
- Erythropoiesis is the process by which a **hemocytoblast** stem cell differentiates into a mature RBC.
- cells are released into circulation as Reticulocytes.
- Hematocrit (HCT or Ht) is the volume percentage of RBCs in the whole blood sample. Normal Adult Values of Hematocrit:
 - Male — 47% \pm 5% (testosterone effect).
 - Female — 42% \pm 5%
- Blood Typing identifies the Antigens on the RBC’s surface.
- The International Society of Blood Transfusion has recognized 39 major human blood type systems as of July 2019. We will look at just two of those systems:
 - **ABO** system — gene on Chromosome **9** (9q34.2)
 - **Rh** system — gene on Chromosome **1** (1p36.11)
 - specifically the **Rh(D)** antigen

□ **Reticulocyte**

- Stage of RBC differentiation just before Mature Erythrocyte.
 - makes up about **1% to 2%** of all the circulating RBCs.
 - takes about **1 to 2 days** to become a Mature Erythrocyte.
 - “*branchy mesh-like clumps of ribosomal RNA*” are visible.
-

□ **Leukocytes** (White Blood Cells)

- Divided into two groups: Agranulocytes & Granulocytes.
- WBCs only make up about 1% of the whole blood volume.
- WBCs are found with platelets in the “*Buffy Coat*”... which is a thin white layer between the RBCs and Plasma of a centrifuged tube of whole blood.

□ Granulocytes:

□ **Neutrophils**

- Percentage in Adults: 50% to 70%
- Diameter: 10µm to 12µm
- Nucleus: 3 to 5 lobes (Multi-lobed)
- Granules: very tiny. light purple or pink.
- Lifespan: 6 hours to a few days.
- Main Duties: Phagocytosis of Microbes.
- Other facts: have the green heme enzyme myeloperoxidase in granules.

□ **Eosinophils**

- Percentage in Adults: 2% to 4%
- Diameter: 10µm to 12µm
- Nucleus: 2 lobes (Bilobed)
- Granules: large. red, orange or dark pink.
- Lifespan: 8 to 12 days.
- Main Duties: Antiparasitic (vs. helminths)
Modulate the allergic response.

□ **Basophils**

- Percentage in Adults: 0.5% to 1%
- Diameter: 12µm to 15µm
- Nucleus: U-shaped or S-shaped.
- Granules: large. blue.
- Lifespan: few hours to a few days.
- Main Duties: release **histamine**:
 - **vasodilation**
 - **bronchoconstriction**

□ Agranulocytes:

□ **Lymphocytes**

- Percentage in Adults: 25% to 45%
- Diameter: small cells: 7µm to 8µm
large cells: 12µm to 15µm
- Nucleus: large, dark, “dent” may be seen.
- Granules: none.
- Lifespan: Years for memory B cells.
Weeks for all others.
- Main Duties: **B cells** - release antibodies
T cells - adaptive responses

□ **Monocytes**

- Percentage in Adults: 3% to 8%
- Diameter: 15µm to 30µm
- Nucleus: large, kidney-shaped.
- Granules: none.
- Lifespan: hours to days.
- Main Duties: phagocytosis.

□ **Platelets (Thrombocytes)**

- formed element of blood that is part of the clotting cascade.
- shape is that of an oblate spheroid.
- have a dark purple color with Wright's Stain
- have no nucleus.
- are just the cytoplasmic fragments of the **megakaryocyte**.
 - *one megakaryocyte can make 1000 to 3000 platelets!*
- the largest platelets are only about 2µm to 3µm in diameter.
 - *this is the same size as a typical large bacteria!!!*
- Lifespan of a typical platelet is about 8 to 9 days.

Notes:

Blood Typing & Performing a Wright's Stain on your own Blood:

*You are not required to perform a blood type and Wright's Stain of your own blood if you don't want to. However, you must still know the information contained here and be able to recognize cells on a blood smear slide (such as Slide 2-3-1) and interpret a blood typing test card. We will only be blood typing the two most critical systems: ABO and Rh.

The Blood Typing & Wright's Stain Procedures:

□ Materials List:

	<u># per student</u>	<u>OK to Share? (Yes/No)</u>	<u>Hazardous Waste* Disposal? (Yes/No)</u>
• one alcohol wipe	1 or 2	NO	YES
• sterile lancet	1	NO	YES
• blood test card	1	NO	YES
• mixing sticks	3	NO	YES
• bandaid	1	NO	YES
• cotton swab	1	NO	YES
• microscope slide	2	NO	YES
• cover slip	1	NO	YES
• Bibulous Paper	1 pad	Yes (one at staining station)	NO
• anti-A serum vial	1	Yes	NO**
• anti-B serum vial	1	Yes	NO**
• anti-D serum vial	1	Yes	NO**
• Wright's Stain	1	Yes (one station for the class)	NO

*Hazardous Waste containers (Red Bag & Sharps Container) will be pointed out in lab.

**serum testing vials are returned to the refrigerator after the lab is over.

NOTE: you will be preparing a Blood Smear Slide at the Same Time as your Blood Typing Procedure.

1. Watch the Professor's Demo first, and note any additional instructions not listed here.
2. Wash your hands with soap and water.
3. Select a finger tip and wipe the site to be lanced with an alcohol wipe.
4. Lance your finger. Be sure to hold the sterile lancet firmly pressed into your finger before you press the button. The lateral side of the finger tip is a good spot to try.
5. Wipe the initial blood drop away with the cotton swab (it is mostly serum anyway).
6. Put 1 drop in the "A" well, 1 drop in the "B" well, 1 drop in the "D" well, and 1 drop onto the microscope slide (about 1/3 the distance from the edge as shown in the demo).
7. Perform the Smear Technique with the spare microscope slide as seen in the demo.
8. Let the Blood Smear slide air dry while you perform the Blood Typing Procedure.
9. Put one drop of the BLUE anti-A serum next to the drop of blood in the "A" well.
10. Mix the BLUE anti-A serum and blood together with a mixing stick.
11. Discard the mixing stick.
12. Put one drop of the YELLOW anti-B serum next to the drop of blood in the "B" well.
13. Mix the YELLOW anti-B serum and blood together with a mixing stick.
14. Discard the mixing stick.
15. Put one drop of the CLEAR anti-D serum next to the drop of blood in the "D" well.
16. Mix the CLEAR anti-D serum and the blood together with a mixing stick.
17. Discard the mixing stick.
18. Rock the Blood Test Card back and forth for 2 minutes to encourage mixing & clotting.
19. Interpret the Blood Test Card. Remember — *"your blood type is what clots!"*

20. Now we will finish the Blood Smear Slide which has had time to air dry.
21. Dip your Blood Smear Slide into the Coplin Jar of Wright's Stain using a wooden clothes pin.
22. Let the slide soak for 45 seconds.
23. Remove the slide and gently rinse with Deionized Water in the sink.
24. Blot Dry just a little bit with the Bibulous Paper pad. Mostly Dry the bottom of the slide... the top needs to still be wet to hold onto the cover slip.
25. Add a cover slip.
26. View the Blood Smear Slide at 400X or 1000X (oil immersion) and look for examples of each cell (Erythrocytes, Platelets, Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes).
27. Discard any Biohazards in the Hazardous Waste containers after you are done.
28. Discard any wrappers and paper trash in the Black Trash Bins.

Blood Type Card Interpretation:

- The easiest rule of thumb to know when interpreting blood type cards is, *“Whatever clots is the Blood Type”*.
- Remember “O” is the absence of A or B antigens on the surface of the RBC.
- Remember “Rh negative” is the absence of the Rh(D) antigen on the surface of the RBC.
- The “Universal Donor” Blood Type is O negative.
- The “Universal Recipient” Blood Type is AB positive.
- When a patient is receiving blood from a donor, they can *“only receive what RBC antigens they already have or less than that.”* A patient can NOT receive antigens they do not have! If they do, then their immune system will begin a Type 2 Hypersensitivity Allergic Reaction and start clotting the donated blood in a few minutes!

Blood Type Compatibility* Chart *Yes = Compatible		Recipient's Blood Type							
		O negative	O positive	A negative	A positive	B negative	B positive	AB negative	AB positive “Universal Recipient”
Donor's Blood Type	O negative “Universal Donor”	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	O positive		Yes		Yes		Yes		Yes
	A negative			Yes	Yes			Yes	Yes
	A positive				Yes				Yes
	B negative					Yes	Yes	Yes	Yes
	B positive						Yes		Yes
	AB negative							Yes	Yes
	AB positive								Yes

NOTE: When writing blood types as answers for a test question, it is best to fully write out the Blood Types like you see in the chart above. “A negative” is much clearer and less likely to be handwritten (or typed) wrong than “A –”. Either way of writing the answer will still be correct though.