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Hematology / Immunohematology (Click on link to access this section)

Hematology Immunohematology

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Hematology / Immunohematology

Blood and Blood Tests

Learning Objectives

- Know the basic blood components (formed elements, plasma)
- Identify the three basic blood cell types (RBC, WBC, platelets)
- For leukocytes, be able to:
  - Identify the different leukocytes in a blood smear
  - Identify special cell features of each leukocyte (i.e., nucleus, granules)
  - Give a general function for each leukocyte
  - Give normal ranges for each leukocyte in a differential
  - Give medical indications for values outside normal ranges
- Be able to read a spun hematocrit and know its significance. Know the normal values for males and females. Know the medical indications and medical terminology for values outside normal range.

1

Introduction

Blood is a liquid connective tissue that is comprised of formed elements (cells) in a liquid (plasma or serum). As you learned in API, there are three basic cell types (WBCs, RBCs, and platelets).

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CBC and Differential

The blood of almost all patients with major illnesses is examined because of the importance of determining the presence of anemia and/or changes in leukocytes. Therefore, one of the most common blood tests done in the laboratory is the CBC (complete blood count) and DIFF (short for differential). The CBC is comprised of WBC (white blood cell), RBC (red blood cell), HGB (hemoglobin), HCT (hemocrit), platelet count, three indicies (MCV, MCH, MCHC), and a white cell differential.

WBC

The normal white cell count is around 4,500–10,000/µL (normal blood cell ranges differ slightly by the instrument used and the population). The cells are then examined to determine the percentage of the various types of white blood cells. The total WBC count is determined by a blood cell analyzer and the different types of WBCs can be determined by the analyzer or a blood smear can be made and stained with Wright's stain. One hundred cells are counted and are divided (differentiated) into the five basic types of cells.

If the white blood cell count is higher than normal (leukocytosis) it may indicate an infection or inflammation, an immune system disorder, or a bone marrow disease. The differential becomes especially helpful in determining the diagnosis.

If the white blood cell count is lower than normal (leukopenia) it is associated with acute viral infections, chemotherapy treatment, and other infections.

RBC

The normal red blood cell count differs between the sexes. For females, it is approximately 3.90–5.03 million cells/µL and 4.32–5.72 million cells/µL for males.

In anemia, the red cell count is lower than normal. Anemia causes fatigue and weakness. Anemia has many causes, including low levels of iron, certain vitamins (e.g., B12), blood loss, or other underlying conditions, such as cancer.

A red blood cell count that is higher than normal (erythrocytosis), or high hemoglobin or hematocrit levels, could point to an underlying medical condition, such as polycythemia vera or heart disease.

PLATELETS

Platelets are fragments of a much larger cell called a megakaryocyte. Platelets are involved in the clotting process and form the initial platelet plug to stop bleeding. A platelet count that's lower than normal (thrombocytopenia) or higher than normal (thrombocytosis) is often a sign of an underlying medical condition, or it may be a side effect from medication.

Note: The above three cells were studied in API Lab and should be reviewed.

PERIPHERAL SMEAR ANALYSIS

To perform a manual differential, a drop of blood is spread thinly onto a glass slide that is then stained. Wright's stain is a mixture of eosin and other dyes, including methylene blue. Eosin is an acidic red-orange dye and structures that take up that dye are referred to as eosinophilic. Structures that take up basic dyes (e.g., methylene blue) are referred to as basophilic. Structures that take up a combination of both dyes are called neutrophilic.

The differential is performed by counting one hundred WBCs on the stained slide. In the past, a blood smear was prepared with every CBC. However, modern automated blood cell counting analyzers are able to perform most differentials. However, if the results from an automated cell count and/or the differential indicate the presence of abnormal white blood cells (WBCs), red blood cells (RBCs), and/or platelets or if there is reason to suspect that abnormal cells are present, then a manual blood smear will be made and read.

A blood smear is used to categorize and/or identify conditions that affect one or more types of blood cells and to monitor individuals undergoing treatment for these conditions. There are many diseases, disorders, and deficiencies that can affect the number and type of blood cells produced, their function, and their lifespan.

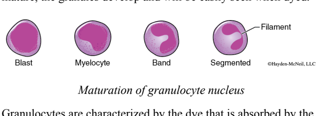
In this lab, you will be required to identify the different types of white blood cells. For the practical exam, only mature WBCs will be chosen. Reading differentials takes time, so be sure to allow more than one lab session to review the blood smears.

IDENTIFYING LEUKOCYTES

Leukocytes are divided into two basic categories, depending on whether there are visible granules in the cytoplasm of the cell. Granulocytes have granules, while agranulocytes generally lack granules (they do have some but they are small and not very conspicuous). Another difference between granulocytes and agranulocytes is the shape of the nucleus of the cell. The size of the cells also differs greatly, so it is important when trying to differentiate the leukocytes, that one observes the size of the cell (as compared to an erythrocyte), the shape of the nucleus, and the color of any granules.

Granulocytes

Young granulocytes are called blasts. Blasts are large cells, with little cytoplasm. As the granulocyte matures, the size of the cell shrinks. The nucleus of the cell, which as a blast is generally round in shape, begins to pinch in to more of a kidney shape. The pinching continues until two lobes are formed with a connecting filament. (**This is the process of segmentation.**) The older the cell, the more lobes that will form. If one observes the nucleus, the blast has a fine texture, while the nucleus of the mature granulocyte will appear denser. As the cell matures the ratio of nucleus to cytoplasm changes and the amount of cytoplasm increases. Agranulocytes do NOT go through this particular maturation process, so if one observes a nucleus with lobes and connecting filaments, the cell is a granulocyte. As granulocytes mature, the granules develop and will be easily seen when dyed.



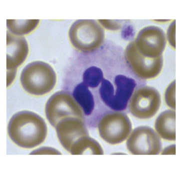
Maturation of granulocyte nucleus

Granulocytes are characterized by the dye that is absorbed by the granules. Eosinophils have a very strong affinity for acid dyes and thus pick up the eosin dye. Since eosin is a fluorescent red dye, the granules appear a bright orange-red. Basophils have an affinity for basic dyes and will pick up the methylene blue and other basic dyes, thus the granules will appear deep purple. Neutrophils take up both dyes, and the granules will appear to not be stained; however, if one closely observes the cytoplasm, lightly staining pink and blue granules will be seen over the entire cytoplasm.

Neutrophils

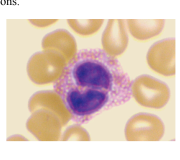
Neutrophils are generally close to twice the size as an erythrocyte (average is around 12 µm). Because of the light staining of the granules, the nucleus is clearly seen. In a normal, healthy individual about half of the neutrophils will have three lobes. Decreased number of lobes (a shift to the left) means that the bone marrow is releasing these cells early. Increased number of lobes (hypersegmentation) is seen in megaloblastic or iron deficiency anemia.

Neutrophils are phagocytes, and are the primary white blood cells that respond to bacterial infections. Because of their immune system function, neutrophils usually make up 50–70% of the white blood cells. In an infection, the bone marrow will respond by releasing an increased number of young neutrophils, resulting in neutrophilia (increased number of neutrophils and increased number of neutrophils with fewer or no lobes). Neutrophilia is also seen in acute inflammation (e.g., appendicitis, burns, heart attacks, etc.).



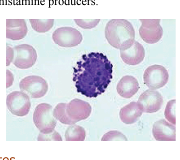
Eosinophils

Eosinophils are characterized by large round or oval granules that pick up the eosin dye, so that they appear red-orange. The nucleus usually stains slightly less intense than a neutrophil and frequently contains only two lobes and rarely more than three. The granules are generally spread evenly over the cell. Normally eosinophils will make up about 3–5% of the white blood cells. An increase in eosinophils (eosinophilia) is often seen in allergies and parasitic worm infestations.



Basophils

Basophils are characterized by large dark granules in the cytoplasm. The nucleus usually is just indented or partially lobed. The granules may cover the entire cell or may be found in a few clusters. Sometimes, the granules may be missing because the granules are soluble in water, which leaves vacuoles (or openings) in the cytoplasm. Basophils are the least common of the white blood cells and it is not unusual to see none in a differential count (normal is 0–1%). Basophilia is seen in myxedema, hypothyroid conditions, ulcerative colitis, certain types of anemia, and cancers. Basophils are histamine producers.

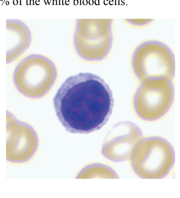


Agranulocytes

Agranulocytes are noted for the absence of granules in the cytoplasm. As noted previously, the nucleus does NOT undergo segmentation as the granulocytes do.

Lymphocytes

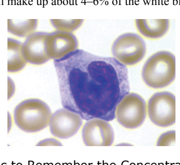
The smallest of the leukocytes, the normal lymphocyte has a large, dark-staining nucleus with little pale blue cytoplasm. In normal situations, the coarse, dense nucleus of a lymphocyte is approximately the size of a red blood cell and is generally round in shape. There are three types of lymphocytes: natural killer cells, T cells, and B cells. Experienced hematologists can differentiate between the different types of lymphocytes, but students are not required to do so. Lymphocytosis is seen in infancy, exophthalmic goiter, mumps, rubella, infectious mononucleosis, sunburn, pertussis, and viral infections. Lymphopenia is associated with increased rates of infection after surgery or trauma as well as chemotherapy treatment. Normally lymphocytes will make up about 25 to 35% of the white blood cells.



Monocytes

The monocyte is the largest cell in normal blood. It is generally two to three times the size of the RBC. The nucleus of the monocyte may be any shape, but is frequently oval or round or indented to form a horseshoe shape. The nucleus stains less densely than other leukocytes and has a looser chromatin pattern than the lymphocyte. The cytoplasm is a blue-gray color and frequently contains tiny red to purple granules. These granules are not to be much smaller and finer than in the granulocytes and should not be confused. Because the monocyte is a phagocyte, the cytoplasm is frequently seen with vacuoles. Monocytes give rise to macrophages and dendritic cells in the immune system.

Because of the monocytes' role in the immune system, increased numbers may be found in many conditions, including autoimmune diseases, gastrointestinal disorders, several viral and bacterial infections, post-splenectomy, inflammatory bowel disease, and some chronic infections such as tuberculosis, syphilis, and Rocky Mountain spotted fever. Autoimmune disorders such as lupus or rheumatoid arthritis might also lead to monocytosis. Normally monocytes will make up about 4–6% of the white blood cells.



**Mnemonic to Remember the Concentration of White Blood Cells

From greatest to least concentration:

- "**N**ever **L**et **M**onkeys **E**at **B**ananas"
- **N**eutrophils, **L**ymphocytes, **M**onocytes, **E**osinophils, **B**asophils

**Mnemonic to Remember Which Cells Are Granulocytes and Agranulocytes

"**BEN** Loves **M**oney":

- Granulocytes: **B**asophil, **E**osinophil, **N**eutrophil
- Agranulocytes: **L**ymphocytes, **M**onocytes

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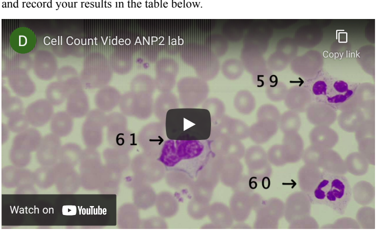
ONLINE Lab Exercise 1

When you see this icon, it means there is something for you to do in your Hayden-McNeil Online course.

Watch supplied videos of a blood smear. The video has been designed to simulate what you would do in lab looking at blood slides. Play the videos and identify each WBC type and keep track of the numbers. Once you have counted 100, fill in the chart. What you have completed is a differential blood cell count! (To calculate %, divide number of cells of each type counted by 100 and then × 100 ((WBC count/100)*100) = WBC%.) Watch the video and complete the fillable version of this table in your Hayden-McNeil Online Course.

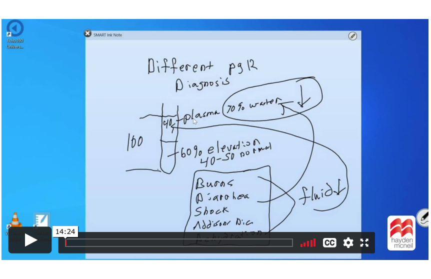
Perform a 100 cell count differential (cell counters are available) and record your results in the table below.



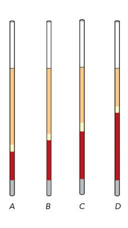
Cell Type	Total	%
Neutrophils		
Eosinophils		
Basophils		
Lymphocytes		
Monocytes		

HEMATOCRIT (HCT)

The hematocrit or packed cell volume is the percentage of the volume of whole blood that is made up of red blood cells. In females, the normal hematocrit is 37 to 47%; for males, 42 to 52%. A lower than normal hematocrit is indicative of anemia, while a higher than normal value indicates polycythemia.



For the following samples (A–D), measure each section of the blood tube (orange, yellow, and red), add them up, and divide the red measurement by the total. That is the hematocrit in %. (Example: total color column height 2 cm, red area 1 cm, 1 cm/2 cm * 100 = 50%)



ONLINE

Test Tube	RBC %
A	
B	
C	
D	

HEMOGLOBIN (HGB)

Unlike hematocrit, which is a relative measure of the RBCs (%), hemoglobin is a direct measure in (grams/dL). The measure of hemoglobin under normal conditions is directly related to the oxygen-carrying capacity of the blood and therefore a measure of how well body tissues are being oxygenated. A commonality between hemoglobin and hematocrit is that they are significantly different between males and females. Male hemoglobin ranges from 13.8 to 18.0 g/dL and females 12.1 to 15.1 g/dL. We will not be performing a lab test for hemoglobin but you are expected to know its basic function/purpose, normal values, and what could cause abnormally high or low values.

DIFFERENTIAL DIAGNOSIS

This is as good a time as any to introduce this concept. If you have watched the popular TV show *House* you have already been introduced to this process. When House is discussing what unique condition the patient has and he is writing all the possibilities on the white board, that is differential diagnosis: all the possibilities you can use to explain the condition your patient is displaying. In this section you will be presented with two differential diagnosis lists, one for low and high hemoglobin and one for hematocrit.

ONLINE Hematocrit Differential Diagnosis

High Hematocrit	Low Hematocrit
Severe Burns	Blood Loss
Diarrhea	Cancers
Shock	Kidney And Liver Diseases
Addison's Disease	Malnutrition
Dehydration	Vitamin B12 And Folic Acid Deficiencies
	Iron Deficiency
	Pregnancy
	Systemic Lupus Erythematosus
	Rheumatoid Arthritis
	Peptic Ulcer Disease

High Hemoglobin	Low Hemoglobin
Anabolic steroids	Vitamin and iron deficiencies
Congenital heart disease	Acute or chronic bleeding
COPD	Hormone deficiencies, such as thyroid hormone or testosterone
Dehydration	Chronic diseases, such as kidney failure, cancer and Crohn's disease
Emphysema	Autoimmune diseases, such as lupus
EPO	Side effect of certain medications, such as chemotherapy drugs
Heart failure	
Kidney cancer	
Living at high altitude	
Other types of heart disease	
Other types of lung disease	
Polycythemia vera	
Pulmonary fibrosis	
Smoking	

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ONLINE Lab Exercise 2

From the lists on the previous page pick three conditions from each of the high and low groups and give a physiological explanation for why that condition would affect Hct/Hgb as it does.

For example: EPO (erythropoietin) is listed under high hemoglobin. Why? The body detects low oxygen levels in the blood through signaling pathways in the kidney. The kidney does not know why oxygen is low, only that it is. The response is to release EPO from the endocrine cells in the kidney. The EPO travels through the blood stream to the bone marrow, where it stimulates hemopoietic stem cells to mature developing into red blood cells. These cells get released into the blood stream, allowing for more oxygen to be transported, which shuts down the feedback cycle that was started in the kidney from low oxygen.

High Hct:

1. ______________________

2. ______________________

3. ______________________

Low Hct:

4. ______________________

5. ______________________

6. ______________________

High Hgb:

7. ______________________

8. ______________________

9. ______________________

Low High:

10.

11.

12.

Blood-Typing

Learning Objectives

- Be able to define the terms antigen and antibody
- For the four basic ABO groups:
  - Know the antigens on the RBC
  - Know the antibodies found in the plasma
  - Understand the transfusion of blood groups (what blood types can donate/receive to which blood types)
- For the Rh type:
  - Know the antigen on the RBC
- Understand the relationship of the RH system and the ABO system in determining a person's "Blood type"
- Understand the cause of hemolytic disease of the newborn
- Be able to "read" a blood-typing slide

Introduction

Blood group systems are inherited features on the surface of red blood cells. A series of related blood types constitutes a blood group system, such as the Rh or ABO system. A **blood type** (also called a **blood group**) is a classification based on the presence of antigens on the RBCs. The two most important blood group systems are ABO and Rh. They determine someone's blood type.

ABO System

The ABO group is determined by the presence (or absence) of A and/or B antigens on the surface of the RBC. The presence of an antigen determines the ABO group (i.e., if there are A antigens on the red cell, then the individual is said to have A blood type). As you learned in General Biology, the ABO system is a codominant system, which means if both the A and B antigens are present, both are expressed.

ABO Group	Antigens Present
A	A
B	B
AB	A and B
O	NONE

The ABO system is unique in that antibodies to the ABO system are present a few months after birth. All other blood group systems do not have "pre-formed" antibodies, as they are caused by exposure by transfusion or pregnancy. Antibodies are formed against the ABO antigen NOT present (if the antibody was against the antigen present, an autoimmune reaction would occur) and circulate in the plasma.

ABO Group	Antigens Present on RBC	Antibodies in Plasma
A	A	ANTI-B
B	B	ANTI-A
AB	A and B	NONE
O	NONE	ANTI-A AND ANTI-B

The pre-formed antibodies become important when blood transfusions are necessary. They must be considered, because if a patient is transfused with incompatible blood (e.g., a person with anti-A in their plasma transfused with blood that has A antigen), it is a life-threatening situation.

TRANSFUSIONS—RECEIVING BLOOD

When blood is required, generally packed red blood cells are transfused. Packed RBCs are made by removing the plasma and platelets from donor blood. This reduces blood volume and the chance of adverse reactions to antibodies and substances that may be circulating in the donor's plasma. Because the plasma and platelets have been removed, just the antigen present on the RBC surface is transfused. This donor antigen CANNOT be the same as the circulating antibody of the recipient (receiver). For example, looking at the chart above, if the donor is an A, the donor blood could not be given to group B or O patients because of their circulating anti-A. Generally speaking, patients can receive their own blood group. If you notice in the chart below, AB can receive from all blood groups (because there are no antibodies circulating in the recipient plasma that could react with donor antigens). This is the basis of the statement "AB is the universal recipient." However, one must understand that the statement only applies to the transfusion of RBCs. Plasma transfusions are the opposite!

ABO Group	Antigens Present on RBC	Antibodies in Plasma	Receive Blood From
A	A	ANTI-B	A, O
B	B	ANTI-A	B, O
AB	A AND B	NONE	A, B, AB, O
O	NONE	ANTI-A AND ANTI-B	O

TRANSFUSIONS—DONATING BLOOD

When donating blood, generally the blood is not given to a specific patient for whom it is donated, except under special cases (donating blood in a patient's name pays for the cost of the unit they are receiving). In rare cases, or in bone marrow transplants, donations are made specifically to the donor. When receiving blood we consider the patient's antibody. If one is donating blood, then the antigen present on the red cell of the donor is looked at. For example: If you are an AB, and are asked if you can donate to a person, then you would consider the antigens present on your cells and what blood type does not possess those antibodies. Since blood groups A, B, and O all have circulating anti-A and/or anti-B, you could donate to only another AB person. If you notice in the chart below, O can be given to all blood groups (because there are no antibodies circulating in the recipient plasma that could react with donor antigens). This is the basis of the statement "AB is the universal recipient." However, one must understand that the statement only applies to the transfusion of RBCs. Plasma transfusions are the opposite!

ABO Group	Antigens Present on RBC	Antibodies in Plasma	Receive Blood From	Can Donate To
A	A	ANTI-B	A, O	A, AB
B	B	ANTI-A	B, O	B, AB
AB	A AND B	NONE	A, B, AB, O	AB
O	NONE	ANTI-A AND ANTI-B	O	A, B, AB, O

In the US, 48% of the population is blood group O. Considering that O can only receive from other Os, you should now understand why blood banks frequently put out the call for O donors!

Rh System

The Rh system is another important blood group. Although there are more than fifty antigens in the system, the one people are most familiar with is the D antigen. The D antigen is a dominant antigen and if you possess at least one D antigen, the Rh type is said to be "positive." Rh negative individuals lack the D antigen. D negative individuals (or Rh negative) can develop anti-D if transfused with D positive blood. D negative blood can be given to both D positive and negative individuals. The Rh system does NOT have pre-formed antibodies.

Rh Type	D Antigen	Can Receive Blood From	Can Donate To
Rh+	present	Rh-, Rh+	Rh+
Rh-	absent	Rh-	Rh+, Rh-

The Blood Type

When referring to the blood type, both the ABO group and the Rh type are included. Thus, if both A and D antigen are found on the RBCs, the blood type is said to be "A positive" and that individual can receive A positive, A negative, O positive, and O negative blood or can donate to A positive and AB positive individuals.

***Fill out the chart below to make sure you understand this concept.**

Blood Type	Antigens Present on RBC	Antibodies in Plasma	Can Receive Blood From	Can Donate To
A+				
A-				
B+				
B-				
AB+				
AB-				
O+				
O-				

Hemolytic Disease of the Newborn (HDN)

Hemolytic disease of the newborn (HDN) or erythroblastosis fetalis is a disease where the mother's antibodies (IgG) cross the placenta and lyse (rupture) the fetus' RBCs. The disease can be mild (as usually found in ABO incompatibility) or severe (usually Rh incompatibility), and fetal death from heart failure (*hydrops fetalis*) can occur. When the disease is moderate or severe, many erythroblasts (early red blood cells) are present in the fetal blood because the bone marrow is trying to replace the destroyed RBCs, thus the name erythroblastosis fetalis.

HDN can occur with an incompatibility of any blood group, but involvement of the ABO and Rh systems is the most frequent. HDN is the most serious when the mother is Rh negative and the fetus is Rh positive (father is Rh positive). Because there are no pre-formed antibodies with the Rh system, the first baby usually does not have a problem. However, childbirth, abortions, ruptures in the placenta during pregnancy, or medical procedures carried out in the pregnancy can cause the mother to be exposed to the fetus' Rh positive RBCs. The mother's immune system will develop anti-D and the antibody will circulate in the mother's plasma. This antibody is small enough to cross the placenta and attack any subsequent pregnancies that are Rh positive.

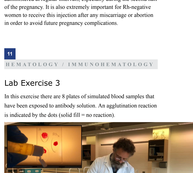
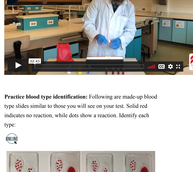
To combat this problem, RhoGAM is given.** RhoGAM is IgG anti-D and the antibodies will lyse any D positive fetal cells before the maternal immune system can react.

Rho is the blood bank's designation for the D antigen and GAM stands for gamma globulin (IgG).

To prevent the development of Rh antibodies, RhoGAM is usually administered during the 28th week of the first pregnancy, and then again within 72 hours after delivery, if the baby is Rh positive. For every subsequent pregnancy after the first, RhoGAM is administered at regular intervals, especially during the second half of the pregnancy. It is also extremely important for Rh-negative women to receive this injection after any miscarriage or abortion in order to avoid future pregnancy complications.

Lab Exercise 3

In this exercise there are 8 plates of simulated blood samples that have been exposed to antibody solution. An agglutination reaction is indicated by the dots (solid fill = no reaction).



Practice blood type identification: Following are made-up blood type slides similar to those you will see on your test. Solid red indicates no reaction, while dots show a reaction. Identify each type: