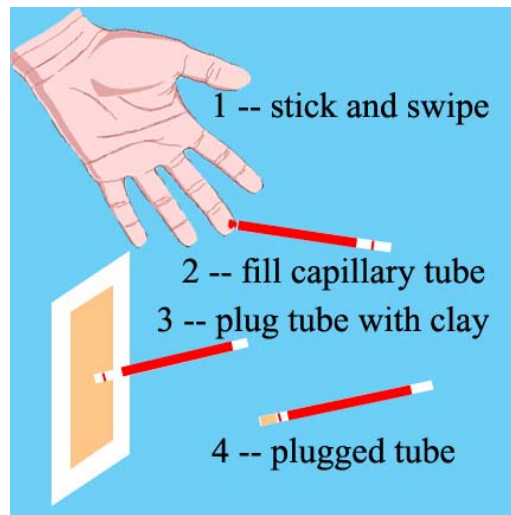


Hematology: Micro-Hematocrit, Hemoglobin and WBC Differential

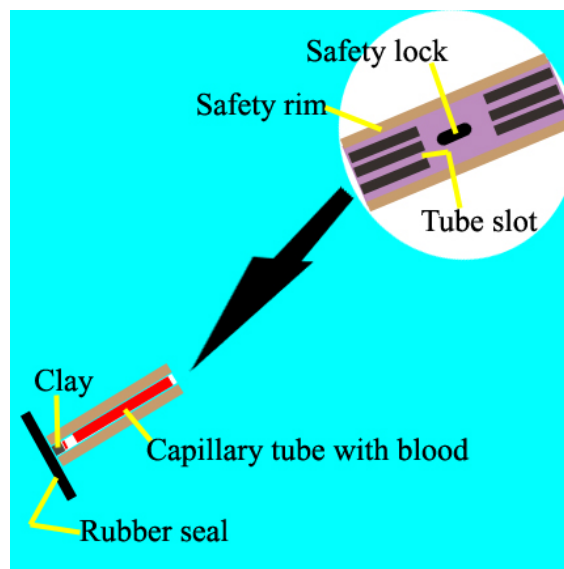
Micro-Hematocrit Determination

The volume of packed red blood cells (pRBC) in blood is called the packed cell volume (PCV) or hematocrit. This value is useful in determining the extent of anemia or hemorrhage in patients with these disorders. Typically, though, these values require some time to adjust to the above events.

In order to perform a micro-hematocrit determination, venous, anti-coagulated blood is allowed to mostly fill a capillary tube that has been coated with heparin, then sealed with clay:

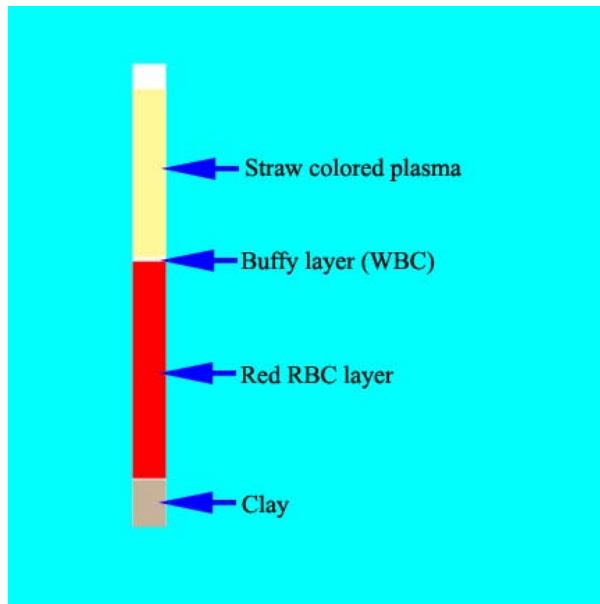


The sealed tube is then laid in a slot in a micro-hematocrit centrifuge CLAY END OUT. These centrifuges come in different styles, but the general idea is represented in the layout below:

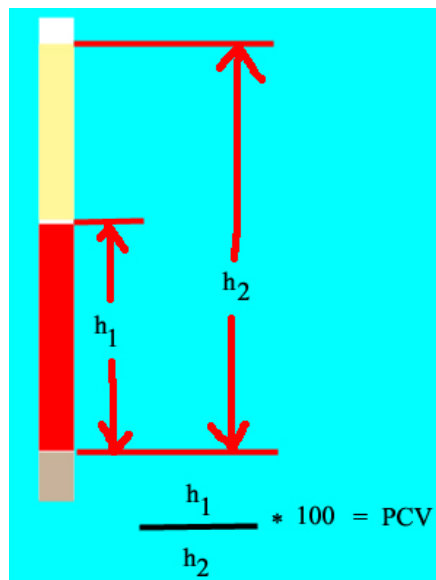


Close the centrifuge lid and latch it. You must hear the second "click" for the centrifuge to run -- if not, it won't. Set the timer for 10 minutes. Observe the centrifuge occasionally to make certain it does not "wander" off the table.

When the centrifuge stops, open to the capillary tubes. Carefully take the tubes out -- without disturbing them. You will notice three layers: on top -- straw colored; on bottom -- red colored; in between -- thin white opaque layer:



The layers contain, respectively, plasma, red blood cells and white blood cells. The hematocrit, or PCV, is determined by measuring the height of the RBC and divide this by the height of the total column of fluid. Multiply this number by 100 to get the hematocrit:



In order for you to accomplish this experiment, have your lab partner perform a finger stick (this will be demonstrated for you by your instructor). Obtain a micro-hematocrit tube and as per the above graphics, perform this determination.

Your Hematocrit: _____%

Hemoglobin

The level of hemoglobin (Hgb) in RBC's is critical for proper O₂/CO₂ exchange in the body. Too little Hgb and not enough O₂ gets to tissues and causes fatigue, among other things. Too much Hgb is also pathologic as it may indicate the inability of normal amounts of Hgb to exchange O₂/CO₂ as in emphysema.

Manual determinations currently approved by the College of American Pathologist (CAP) require the use of various cyanide reagents. The biology lab is not set up to deal with cyanide emergencies; hence, you will not have the opportunity to perform this determination. There is, however, a general rule of thumb that you may remember: the hematocrit is approximately 3 times the Hgb. For example, a hematocrit of 42% corresponds to a Hgb of APPROXIMATELY 14 mg%.

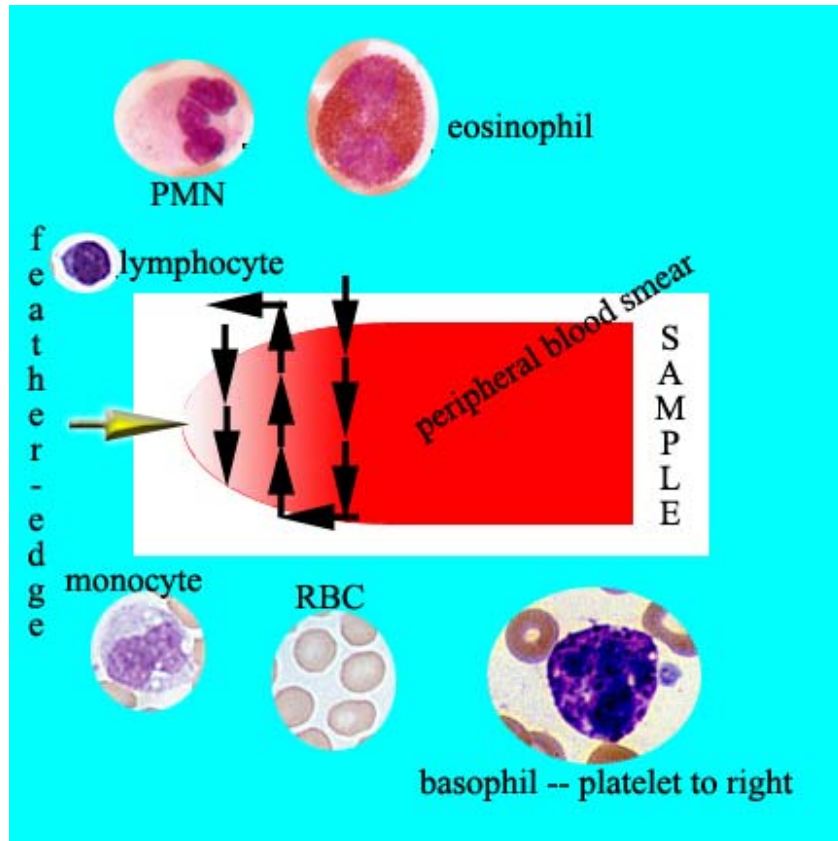
Based upon your hematocrit, what is your approximate Hgb? _____ mg%

White Blood Cell Differential

The white blood cell (WBC) differential (diffy) is microscopic look at how the body is responding (or not) to some type of insult to homeostasis. A drop of blood is smeared on a microscope slide in a bullet-shaped with a fine feather edge. The smear is then stained by one of several modifications of the Wright Stain method. 100 WBC are counted, dividing them into the agranular and granular groups of leukocytes, Table 1, i.e., lymphocytes and monocytes; neutrophils, eosinophils and basophils, respectively.

Table 1	
Agranular WBC	Comments
Lymphocyte	small cytoplasm; nucleus about the size of a RBC
Monocyte	lot's of dirty cytoplasm; nucleus about 3 times the size of RBC
Granular WBC	
Neutrophil*	salmon pink granules; purple segmented nuclei [#]
Eosinophil*	red-orange granules; purple segmented nuclei
Basophil*	blue-black granules; purple segmented nuclei
* all about 2 times the size of a RBC	
[#] immature forms have horse-shoe nucleus -- called "bands" or "stabs"	

The following graphic (magnifications are slightly off for illustrative purposes) illustrates both the direction of microscopic study (per below) AND the kinds of cells you will observe:



In order for you to accomplish this experiment, make your slide as demonstrated by your instructor after you have filled your micro-hematocrit tube. The staining protocol to be used in this experiment is as follows (and may be modified as necessary depending on the age of the reagents):

- Place your dry blood smear in the slide (stain) tank containing the Wright Stain. Leave it there for 10 minutes.
- ASIDE: this tank contains methyl alcohol (wood alcohol). Methyl alcohol is poisonous. Remove slides with forceps.
- After 10 minutes, remove your slide from the stain tank with forceps.
- Place the slide in the next tank and let stand for 5 minutes or until a greenish scum floats over the top of the liquid.
- Remove your slide and rinse under a gentle stream of water from the sink.
- Blot dry with bibulous paper.

- Put under the low power objective on your microscope, focus on the feather edge where the RBC just touch each other (edge-to-edge), go up to high the high power objective and refocus with the fine focus knob.
- Swing the objectives half-way between 40X and 100X objectives -- DO NOT MOVE THE STAGE, AT ALL
- Place 1 drop of immersion oil on the stained smear over the light and swing the 100X objective INTO the oil until it clicks into place.
- Refocus with the fine focus and begin looking at your slide for the WBC. Do this systematically, i.e., one field at a time, width of the slide (per the above graphic with the blood cell types in it).
- Examine the slide for WBC's and record the numbers of each type (Table 1 and lecture notes).
- If time and staining technique permit, count up to 100 WBC and record below:

% segmented neutrophils
% banded neutrophils
% eosinophils
% basophils
% lymphocytes
% monocytes

- If time does not permit, observe at least three "trips" across the slide, one field at a time.
- Remove the slide and dispose of it as directed by your instructor.
- Wipe off the oil from the 100X objective with lens paper and
- Return the microscope to the cabinet.
- Dispose of your blood-collecting waste as before in the sharp's container.