

BLOOD CELL COUNTS: ERYTHROCYTES

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David B. Fankhauser, Ph.D.

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See related protocols: *Blood typing, Hematocrit.*

http://biology.clc.uc.edu/fankhauser/Labs/Anatomy_&_Physiology/A&P202/Blood/RBC_Blood_Counts.htm

SAFETY NOTE: Avoid infection with blood-borne pathogens such as HIV and hepatitis B, by using protective gloves when handling other peoples' blood. Dispose of the blood-contaminated sharps in the marked container, and the rest as indicated by the instructor.

Sufficient numbers of red blood cells (RBC, or erythrocytes) are necessary for adequate transport of oxygen from the lungs to the peripheral tissues. Too few RBCs constitutes a pathological condition known as anemia (lit., "without blood"). According to the Merck Manual, normal values of RBC/cmm for males is 5.4 ± 0.8 million, and 4.8 ± 0.6 for females. Anemic levels for adult males are below 4.5 million, for females below 4.0 million. We will perform red and white blood cell counts on your blood in the lab using a hemacytometer and appropriately diluted blood.

PRACTICE: You should have performed a yeast cell count last week using the same manipulations as follows. Review your notes to be aware of problematic steps.

PRELIMINARIES: Practice again finding the appropriate fields in the hemacytometer for the counts as illustrated at the bottom of the WBC protocol. Review the four illustrations you made last week:

- 1) the entire hemacytometer grid. *Note which squares used for counting RBC, or for WBC.*
- 2) a close up of a single WBC field showing clearly the number of lines surrounding each field
- 3) a close up of a single RBC field showing clearly the number of lines surrounding each field
- 4) dilution pipets and their use.

RED BLOOD CELL COUNT:

1. Swab the tip of a little-used finger with 70% EtOH.
2. Lance with an "autolet" to the side of the pad of the finger, wipe away first blood.
3. Using the dilution pipet with **RED** mixer from hemacytometer kit, draw blood up to the 0.5 mark. This is best done by *slightly* slanting the pipette to allow blood to flow in. Only slight suction should start it. (Make sure the hose is not kinked shut.) *Keep the pipette level* once you have filled it. *Do not allow blood to congeal in pipette!* Immediately proceed to the next step:
4. "Dip and suck": draw **Ringer's solution** diluent up to the 101 mark. (Dilution of 1 to 200.)
5. Shake well to mix with both ends of the pipet sealed.
6. Empty ~1/2 of pipet into waste container. Add a *small* amount of the diluted blood to one chamber of the hemacytometer. It should flow in to fill. (Do not over fill).
7. Let the preparation sit for a minute (for cells to settle). (Meanwhile you may do the WBC assay.)
8. Center the grid at 100x, switch to **400x** and count five fields of 16 smallest squares RBCs with a clicker (fields: top R & L, bottom R & L, center). Include in the count all cells touching left and bottom sides, ignore cells touching top and right sides.
9. Wash out the pipette thoroughly with soap and water, rinse well, finish with distilled H₂O rinse, replace in case. Rinse the mouth pieces in Everclear, rinse with water, replace in case.
10. Calculate the RBCs/cmm: sum the 5 groups, multiply by 10,000 (i.e., add four zeros)

SOLUTIONS AND EQUIPMENT FOR BLOOD COUNTS (Equipment on previous page)

Ringer's Solution, 100 mL:	860 mg NaCl	Diluent for white blood cells:	10 mg crystal violet
(for RBC dilution)	30mg KCl		1.0 ml glacial acetic acid
	35mg CaCl ₂		<i>q.s.</i> to 100 mL with dH ₂ O
	dissolve in dH ₂ O and <i>q.s.</i> to 100 mL.		