

See previous protocols: *Hematocrit Protocol* and *Blood Cell Counts: Erythrocytes*

NOTE: Perform the WBC count first because it requires more blood. Then do the RBC count.

White blood cells (WBC), or leukocytes, are involved in fighting infections and clearing away dead cells. During infections, their numbers increase dramatically above the normal range of 4,800 to 10,800 WBC/cu mm. Finding WBC content above 10,800 is suggestive of infection, or other problems (such as leukemia).

These cells are without pigment, and must be stained to be counted. Therefore, the diluent contains crystal violet for this purpose. It also causes the lysis of RBC so they are not visible. Note that a different dilution pipette is used than that in the RBC technique.

**REVIEW EXERCISE FOR WBC COUNT:**

- 1) Review illustrations of dilution pipets from last week, their use and dilution factors.
- 2) Practice drawing diluent up in the pipet to the 0.5 then to the 11 marks.
- 3) Blow out all diluent before drawing up blood in the actual protocol

**PROTOCOL (These steps are similar to RBC, read that protocol carefully):**

1. Swab the tip of a little-used finger with 70% EtOH.
  2. Lance with quick, firm jab to the side of the pad of the finger, wipe away first blood.
  3. Using the dilution pipet with **WHITE** mixer, draw blood up to the 0.5 mark. This is best done by *slightly* slanting the pipette to allow blood to flow in. 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. Fill the pipet to the 11 mark with **crystal violet diluent**<sup>1</sup> without contaminating the diluent.
  5. Seal the hose end with your finger, wipe off drip from tip, shake well to mix.
  6. Empty ~1/2 of pipet into waste container, apply a small amount of the diluted blood to the second chamber of the hemacytometer. It should flow in under the cover slip to fill the chamber. (Do not over fill).
  7. Let the preparation sit for a minute (for cells to settle).
  8. Examine under **100x**, count and record the five fields (indicated squares) of blue-stained WBCs with a clicker (fields: top L & R, bottom L & R, center). Include in the count all cells touching left and bottom sides, ignore cells touching top and right sides.
  9. **CLEAN UP THE EQUIPMENT:** Wash out the hemocytometer, pipettes and mouth pieces thoroughly with soap and water, rinse well, finish with distilled H<sub>2</sub>O rinse, replace in case. Replace along with two pieces of hose in the case, return to the proper location in the drawer.
  10. Calculate the WBCs/cmm: sum the 5 groups, multiply by 40.
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Illustration of grids for WBC and RBC counts:

