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

See related protocols: *Blood typing, Hematocrit.*

EQUIPMENT

- one Hemacytometer kit per student:
 - Hemacytometer with cover slip
 - WBC diluting pipet (white), hose and mouthpiece
 - RBC diluting pipet (red), hose and mouthpiece
- 10 mL sample beaker, 1 bottle/desk
- 50 mL waste beaker, 1 bottle/desk

SUPPLIES

- two yeast suspensions to be distributed:
 - 1 pkg/100 mL (simulates RBC)
 - 1:1000 dil of above (simulates WBC)
- WBC diluent, 1 bottle/desk
- RBC diluent, 1 bottle/desk
- half a paper towel

Blood cell counts can be performed using the hemacytometer. This is a precision instrument which possesses a platform with microscopic grid scoring above which a specified quantity of fluid is held. By properly diluting blood, counting all cells in specified squares, and multiplying by the proper conversion factor, the number of cells per cubic millimeter can be determined.

Because of the potential dangers of working with blood, we will first practice the necessary dilutions and use the hemacytometer to count yeast cells. Be certain to master these skills before you attempt to do the blood work.

- Illustrate:*
- 1) the grid on the hemocytometer, circle all squares counted for WBC and for RBC
 - 2) vertically aligned top and side views of the hemocytometer (show the cross section)
 - 3) and 4) the dilution pipets: the one used for WBC and the one used for RBC.

Explain their use and what the dilution factors would be. **Immediately after use**, wash out the pipettes thoroughly with soap and water, rinse well with distilled H₂O, replace in case. Same for hemacytometer.

Cut here:

FIRST: Practice drawing respective diluents up in the pipets to the desired volume several times.

PRACTICE: RED BLOOD CELL COUNT PROCEDURE:

1. Get small sample of **concentrated yeast** in your 10 mL beaker from stirring hot plate.
2. Using the RBC dilution (pipet with **RED** mixer), test it by blowing into yeast. (No bubbles = stopped up.) Draw well-mixed concentrated yeast just past the 0.5 mark with slight suction. (Make sure the hose is not kinked shut.) Hold pipet horizontally, touch tip with towel to draw level down to the 0.5 mark. *Keep the pipette level* once you have filled it. Immediately proceed to the next step:
3. Holding pipet nearly level, draw **Ringer's solution (clear)** diluent up to the 101 mark. (Dilution of 1 to 200.) *Do not contaminate the diluent by leaking yeast into the bottle!*
4. Seal tip with your finger and shake well to mix.
5. Empty ~1/2 of pipet into waste container, then add a *small* amount of the diluted yeast to one chamber of the hemacytometer. It should flow in by capillary action to fill. (Do not over fill).
6. Let the cells to settle for a minute. Meanwhile, rinse out the RBC dilution pipet.
7. Center the grid at 100x, switch to **400x** and with a clicker, count and record separately five RBC fields (each with 16 smallest squares) (Record fields: UR, UL, center, LR and LL).
8. Calculate the number of yeast in a cubic millimeter: sum the 5 groups, multiply by 10,000 (i.e., add four zeros).
9. How many cells in the entire package? Calc: $(100 \text{ mL/pkg}/10^{-5} \text{ cmm}/100\text{mL}) \times \text{cells/cmm}$

PRACTICE: LEUKOCYTE CELL COUNT PROCEDURE:

1. Empty the concentrated yeast from 10 mL beaker, rinse well, get small sample of **diluted yeast**.
2. Using WBC dilution pipet (with the **WHITE** mixer), draw yeast just past the 0.5 mark. Pinch hose to close off. Hold horizontally. Dab with piece of paper towel to draw volume down to 0.5. Proceed immediately to the next step:
3. Holding pipet nearly level, fill the pipet by gentle suction to the 11 mark with **crystal violet diluent**¹.
4. Seal the tip with your finger, shake well to mix.
5. Empty ~1/2 of pipet into waste container, then add a *small* amount of the diluted yeast to one chamber of the hemacytometer. It should flow in by capillary action to fill. (Do not over fill).
6. Let the cells to settle for a minute. Meanwhile, rinse out the WBC dilution pipet.
7. Examine under **100x**, count the indicated five square fields of blue-stained yeast with a clicker (Record fields: UR, UL, center, LR and LL.)
8. Calculate the WBCs/cmm: sum the 5 groups, multiply by 40. (Should be about 8,600 cells/cmm)
10. **CLEAN UP THE EQUIPMENT!:** Wash out the 2 pipettes, 2 mouth pieces, 2 pieces of tubing and the hemacytometer thoroughly with soap and water, rinse well, finish with a distilled H₂O rinse, replace in case, return to the proper storage location.