Eosinophil Count

Principle

The eosinophil count is performed by diluting whole blood with a staining solution. The phyloxine B present in the diluting fluid stains only the eosinophils red; all other leukocytes are preserved but not stained.\textsuperscript{1,2,3} The diluted specimen is charged onto a hemacytometer for counting. Using the low-power (10x) objective, the eosinophils appear bright orange-red and are clearly distinguishable from neutrophils, basophils, lymphocytes, and monocytes, which do not stain.

Reagents and Equipment

1. Two eosinophil Unopette reservoirs; each containing 0.755 mL of diluent:
   - Phyloxine B (selective stain for eosinophils) 1 g
   - Propylene glycol 500 mL
   - QS with distilled water to 1 liter
2. Two Unopette capillary pipets, 25 µL
3. Fuchs-Rosenthal hemacytometer with cover glass: two counting areas (4mm x 4mm x 0.2 mm or 3.2 µL volume)
4. Petri dish with filter paper
5. Hand counter
6. Microscope

Specimen

Whole blood, anticoagulated with EDTA, or free-flowing capillary blood may be used.

Procedure

1. Prepare two eosinophil Unopettes as follows:
   a. Using the protective shield on the capillary pipet, puncture the diaphragm as follows:
1) Place reservoir on a flat surface. Grasping the reservoir in one hand, take the pipet assembly in the other hand and push the tip of the pipet shield firmly through the diaphragm in the neck of the reservoir, then remove.

b. Remove the shield from the pipet assembly with a twist and fill the capillary pipet with whole blood. Transfer the whole blood to reservoir as follows:
   1) Wipe excess blood from the outside of the capillary pipet, making certain that no blood is removed from the capillary bore.
   2) Squeeze the reservoir slightly to force out some air. Maintain pressure on the reservoir.
   3) Cover opening of overflow chamber of the pipet with your index finger and seat the pipet securely in the reservoir neck.
   4) Release pressure on the reservoir. Then remove your finger from the pipet opening. Negative pressure will draw the blood into the diluent.
   5) Squeeze the reservoir gently two or three times to rinse the capillary bore, forcing diluent into, but not out of, the overflow chamber, releasing pressure each time to return the mixture to the reservoir.
   6) Place your index finger over the pipet opening and gently invert several times to thoroughly mix the blood with diluent.
   7) Let stand for 10 minutes to allow eosinophils to stain.

2. Clean the hemacytometer and cover glass by flooding them with 70% alcohol. Dry thoroughly with gauze or tissue; do not allow the alcohol to dry on the hemacytometer. Be sure to remove all lint. Place the cover glass in position over the ruled area.

3. Following incubation, mix diluted blood thoroughly by inverting reservoir to resuspend cells. Charge hemacytometer as follows:
   a. Convert to dropper assembly by withdrawing the pipet from the reservoir and reseating it securely in its reverse position.
   b. Clean the capillary bore by inverting the reservoir, gently squeeze the sides and discard the first three or four drops.
   c. Place the pipet tip on the edge of the ruled area of the counting chamber. Carefully charge the hemacytometer with diluted blood by gently squeezing the sides of the reservoir to expel the contents until the chamber is properly filled.
   d. Repeat procedure to charge the other side of the hemacytometer with the first Unopette reservoir.
   e. Place the hemacytometer on moistened filter paper in a Petri dish, and allow to stand 10 minutes to permit the cells to settle.
   f. Using this same procedure, charge a second hemacytometer with the second Unopette reservoir.

4. Carefully place the hemacytometer on the microscope stage. Perform cell count as follows:
   a. With the low-power (10x) objective, locate the ruled area and the upper-left large square.
Eosinophils appear bright orange-red.

b. Eosinophils are counted in the entire ruled area following standard counting procedure.
c. Repeat this counting procedure for the other side of the hemacytometer.
d. Record the counts for each side of the hemacytometer.
e. Count the eosinophils on the second hemacytometer following the above counting procedure.

Calculations

The same general formula used to calculate the leukocyte count is also applied for the eosinophil count. The variation will be in the dilution and volume factors (for the Fuchs-Rosenthal counting chamber). For eosinophil counts, the dilution is 1:32 and the volume counted is 6.4 µL. Calculate the eosinophil count for each Unopette and average the result (x 10⁹/L or /mm³).

Eosinophils/L = # of eosinophils counted X correction for dilution (32) X correction for volume of the two chambers (1/6.4 µL) X 10⁶

Reference Interval

Adults: 0-0.45 x 10⁹/L
Birth: 0-1.5 x 10⁹/L
6 months: 0-0.9 x 10⁹/L
4 years: 0-0.7 x 10⁹/L

Comments

1. Eosinophil counts should be performed within one hour following preparation of the Unopette dilution.

2. The coefficient of variation (CV) is 10% in a 100 cell count.

3. There are three different types of counting chambers available for use in the eosinophil count.
   a. Fuchs-Rosenthal hemacytometer with a total volume of 3.2 mm³
   b. Speirs-Levy hemacytometer with a total volume of 2 mm³
   c. Hemacytometer with Neubauer ruling. This hemacytometer is not recommended, however, because of its relatively small volume (0.9 mm³).
4. Eosinophil counts should be performed with fresh whole blood due to increasing eosinophil fragility with time.

5. Technical sources of error in hemacytometer cell counts are given in Web Table 7-4.

References

