MANUAL PLATELET COUNT

Principle

Whole blood is diluted with a 1% ammonium oxalate solution. The isotonic balance of the diluent is such that all erythrocytes are lysed while the leukocytes, platelets, and reticulocytes remain intact. The standard dilution for platelet counts is 1:100. This dilution is prepared using the leukocyte/platelet Unopette system. The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on a hemacytometer. The cells are allowed to settle and then are counted in a specific area of the hemacytometer chamber under the microscope. The number of platelets is calculated per µL (x 10⁹/L) of blood.

Reagents and Equipment

1. Two leukocyte/platelet Unopette reservoirs; each containing 1.98 mL of the following diluent:
   - Ammonium oxalate 11.45 g
   - Sorensen’s phosphate buffer 1.0 g
   - Thimerosal 0.1 g
   - QS with distilled water to 1 liter
2. Two Unopette capillary pipets, 20 µL
3. Hemacytometer with cover glass
4. Petri dish with filter paper
5. Hand counter
6. Microscope

Quality Control

Commercial quality control materials with established control limits should be run periodically. The frequency is determined by each laboratory's workload. For instance, quality control material may be run at the beginning of each eight-hour shift.

Specimen

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

1. Prepare two leukocyte/platelet 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 erythrocytes to hemolyze.

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 center large square (1 mm²). Examine the entire center square for even distribution of platelets, then carefully switch to the high-dry-power (40x) phase objective for counting platelets. With phase microscopy, platelets appear as round or oval bodies.

b. Platelets are counted in the entire center large square (1 mm²) (Figure 7-7) as follows:

1) Count the platelets in the first row of squares going from left to right, then from right to left in the second row; follow this pattern until all rows are counted.

2) Within each square, count all platelets touching the top and left-hand borders. Do not count any cells touching the bottom or right-hand borders.

3) Use the fine adjustment knob to focus up and down to identify the platelets.

c. Repeat this counting procedure for the other side of the hemacytometer.

d. Record the counts for each center square. The difference between these two counts should not exceed 10%.

e. Count the platelets on the second hemacytometer following the above counting procedure.

Calculations
1. The calculation formula for hemacytometer cell counts determines the number of cells within 1 µL (1 mm³) of blood (Figure 7-9). To make this determination, the total number of cells counted must be corrected for the initial dilution of blood and the volume of diluted blood used. The standard dilution of blood for platelet counts is 1:100; therefore the dilution factor is 100. The volume of diluted blood used is based on the area and depth of the counting area. The area counted is 2 mm² and the depth is 0.1 mm; therefore the volume factor is 0.2 mm³.

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\text{Total number of cells counted} \times \text{dilution factor} \times \frac{1}{\text{volume factor}} = \text{cells/mm}^3
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\text{Cells/mm}^3 = \text{cells/µL} \quad \text{or} \quad \text{cells/µL} \times 10^3 \muL/L = \text{cells} \times 10^9/L
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Example: \(100 \times 10^3 \text{ platelets/µL} \times 10^3 \muL/L = 100 \times 10^9 \text{ platelets/L}\)

2. Average the platelet counts from the duplicate pipets and report result (x 10⁹/L or /mm³).

Reference Interval

\(150-440 \times 10^9 /L\)

Comments

1. Platelet counts should be performed within three hours after the dilution has been prepared.

2. The coefficient of variation (CV) for the 95% confidence limits is ± 22%.

3. If blood is collected by a skin puncture, carefully remove the first drop of blood and collect free-flowing blood for the platelet count. This will minimize the occurrence of platelet clumping and adhesion of platelets to the puncture site.

4. If clumps of platelets are seen in the hemacytometer, the procedure should be repeated. Clumps may be due to inadequate mixing of blood or to poor technique in obtaining the blood specimen.

5. A Wright's-stained peripheral blood smear should be examined and the platelet estimate determined to confirm the hemacytometer platelet count. The platelet estimate should correlate with the platelet count ± 25%. If a discrepancy exists, the platelet count and peripheral blood smear estimate should be repeated.

6. In acute leukemia, there is an increase of blast cells in the peripheral blood. Often fragments of cytoplasm about the size of platelets break off the blast cells. These cytoplasmic fragments are called hyaline bodies. They are the same size and density as platelets and may be counted as platelets by the hemacytometer or automated methods. It
is very important that all platelet counts be confirmed by slide examination so that a false increase of platelets (due to the counting of fragments) is not reported.

7. Platelet satellitism will result in falsely decreased platelet counts. With platelet satellitism, platelets adhere to neutrophils when the blood sample is anticoagulated with EDTA and is not free to be counted. Platelet satellitism can be corrected by redrawing the blood sample using sodium citrate as the anticoagulant. The resulting platelet count must be multiplied by 1.1 to account for the dilutional effect of the citrate anticoagulant.

8. A second method for manual platelet counts is the Rees Ecker method. Using the erythrocyte diluting pipet, whole blood is diluted with a solution containing brilliant cresyl blue, which stains the platelets a light bluish color. The platelets are then counted using a standard hemacytometer and bright field microscopy.

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

References
