To: hematology

Manual erythrocyte count

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There are 2 methods to count the erythrocutes in a (EDTA) blood sample. The easiest and most accurate way is by using an automated erythrocyte counter, usually incorporated in a total blood cell counter.
if such a machine is not available, manual eruthrocyte counting can be done following the method described below.

TOTAL ERYTHROCYTE COUNT (MANUAL METHOD)

Erythrocyte (Gr. erythros, red; kytos, cell) or red blood corpuscles are circular, anucleated, highly flexible, biconcave disc-shaped cells with high edges. The central part is thinner than the outer circle.

Equipment

Blood-mixing pipet (with red mixing stone)
Hemocytometer with cover glass, compound microscope (counting chamber).

Blood-mixing pipet

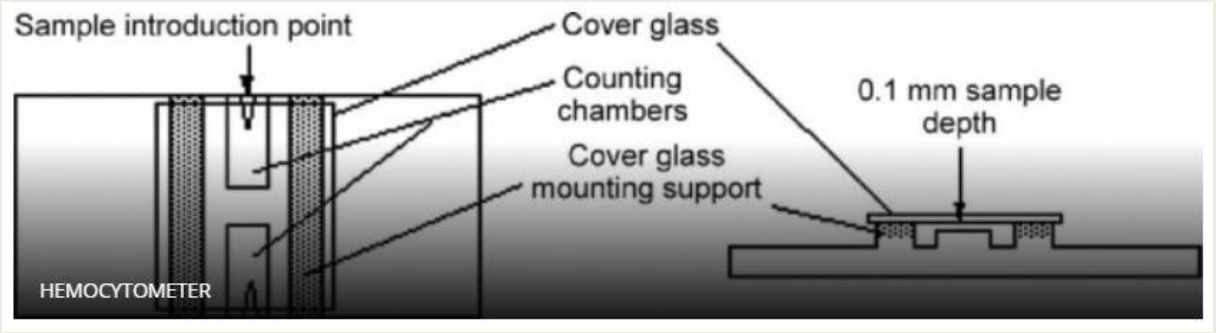
Reagent

Hayem's diluting solution is prepared as follows:

- HgCl2 0.05 gm
- NaSO4 2.5 gm
- NaCl 0.5 gm
- Distilled water 100 ml

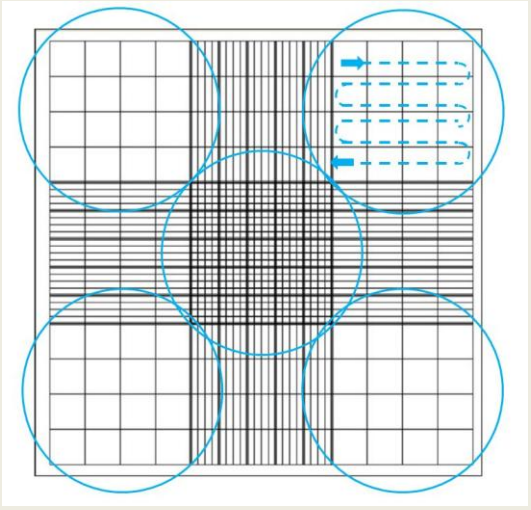
Specimen

EDTA anticoagulated venous blood or blood obtained by skin puncture is used.



Hemocytometer with cover glass

Hemocytometer with cover glass (random choosen commercal sample)


Counting the stained erythrocytes in 80 smallest squares, grouped in 5 middel-sized squares

Method

1. Mixe the blood carefully in its container (without shaking it).
2. Aspirate blood to the 0.5 mark in the blood-mixing pipet..
3. Aspirate diluting Hayem's solution to the 101 mark. It will give 1:200 dilution of the blood.
4. Hold the pipette horizontally and role it with both hands between finger and thumb.
5. Place the counting chamber, absolutely free from dust and grease, on the table and lay the cover glass in place over the ruled area.
6. Discard the first two or three drops from the pipette. Charge the counting chamber by holding the pipette in an inclined position. Allow 3 minutes for the cells to settle.
7. Locate the central square, which is divided into 25 medium sized squares. Each of the medium sized squares is further divided into 16 smallest squares.
8. Count the erythrocytes in medium sized squares (80 smallest squares) using high power objective.
9. In order to avoid confusion in counting, count all cells wihich touch the upper and left outer double line of the group of 16 squares as if they were inside the square. Neglect all those cells, which touch the lower and right inner line.
10. Add 4 zeros to the total number of counted erythrocytes to get the number of erythrocytes in microliters.

Calculation background

Supose number of erythrocytes counted in 5 intermediate squares = E
Area of each of the five squares in which cells are counted = 1/25 sq mm
Therefore, total area counted = 1/25 sq mm x 5 = 1/5 sq mm
Depth of chamber = 1/10 mm
So, the volume in which cells are counted = Area x Depth = 1/5 sqmm x 1/10 mm = 1/50 cu mm
Now, in 1/50 cu mm of diluted blood, the number of erythrocyte counted = E
Number of erythrocyte in one cu mm in diluted blood = E x 50
Since the dilution of the blood is 1 in 200, the number of erythrocytes in one cu mm of undiluted blood = E x 50 x 200

Reference: TOTAL ERYTHROCYTE COUNT (MANUAL METHOD) (bioscience.com.pk)