Lab 6 Hematology

Red Blood Cell (RBCs) count

The red blood cell count on the routine CBC is the concentration of red blood cells, expressed in millions/ μ L of whole blood. While red blood cell counts can be performed by manual techniques, such as a hemocytometer, these are time-consuming and inaccurate.

Counting of RBCs can be performed either:

- 1. Automated method (electronic hematology cell counter e.g coulter)
- 2. Manual method (visual using a microscope such counting chamber)

Hemocytometer:

- Thick glass slide with H shaped moats in it.
- The area between two lines of H shaped is 0.1 mm in depth.
- Moat prevent mixing of two samples on either side of chamber.

Reagent and instruments:

- 1. Neubauer chamber (hemocytometer) and coverslips
- 2.RBCs diluting fluid /solution that consist of normal saline

Method

- 1. Prepare plastic tube (labeled).
- 2. Prepare 1:200 blood dilution (4ml of diluent + 20µl blood).
- 3. Add dilution to the tube and mix the sample 5 times then aspirate 20ul and transfer to the tube and mix
- **4.** Clean the hemocytometer and coverslip with 70% ethanol followed by D.W. and leave it to dry.
- 5. Place the coverslip on the chamber
- 6. Fill the chamber with diluent ($10\mu l$) in each side.
- 7. Place chamber on microscopic stage. Start with 10X to focus; then with 40X count RBCs.

How to count RBCs?

- RBCs should be counted in the central square of the chamber.
- Select 5 small squares (on at each corner and one in the center)

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Count all the cells in specified squares and multiply by the proper conversion factor the number of the cells per cubic millimeter can be determined.

Count all cells within 16 squares and do not count those lying on middle lines.

Calculation:

Total RBC count=N×D/ area×depth

= $N \times 10000$ cells/ μ or mm³

N= numer of cell counted

D(dilution) = 200

Area (Number Large Squares Counted)=1/5

Depth=0.1

Normal Range

• In Adult male: 4.5-5.5 million/mm³.

• In adult female : 3.8-5.2 million/mm³.

• In Infants :6-8 million/mm³

Count of RBCs increase in these cases

- Physiological: new born and infants
- Pathological:

1-polycythemia

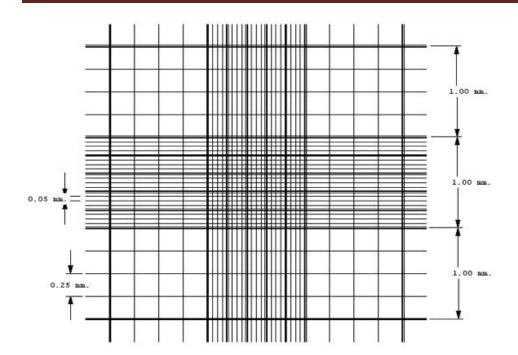
2-dehydration

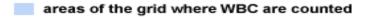
3-hypoxia

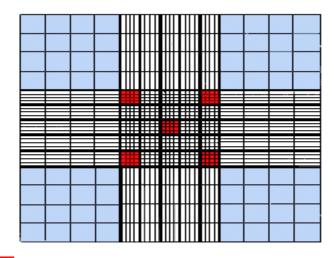
Count of RBCs decrease in these cases:

1-Anaemia

2-pregnancy







areas of the grid where RBC are counted