A red blood cell count is typically ordered as part of a complete blood count (CBC). It is used as a screening test for anemia and polycythemia.

## Manual Red Blood Cell Count (Hemocytometer)

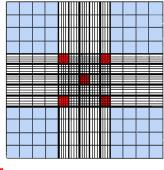
- 1. Obtain 2-5 mL of whole blood in a EDTA tube. Place the specimen in a biohazard bag.
- 2. There are two methods for counting RBC.
- I. Electronic cell counting (coulter count, Hemalog, and Fisher autocytometer) and
- II. Manually cell counting (Hemocytometer).

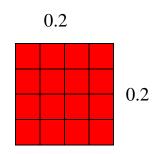
RBC Diluting Fluid (Hayems Solution) is used for diluting RBC's. Contains Sodium Sulfate 2.5 gm, Sodium Chloride 0.5 gm and Mercuric Chloride 0.25 gm in 100 ml D.W. Settling time is between 1 to 3 minutes.

# Procedure

- 1. Obtain 2-5 mL of whole blood in a EDTA tube
- 2. Draw blood to the 0.5 or 1 mark in the RBC Thoma pipet to obtain 1/200 and 1/100, respectively
- 3. Or Pipette 4 ml of RBC diluents in a tube and then Pipette 20  $\mu$ l of well mixed blood into the tube containing the diluents (to obtain 1/200 dilution).
- 4. lean outside of pipette and wash out the blood in the tube containing the diluent (dil 1/200).
- Mix the contents of the tube, and expelled the first few drops of diluted blood from the stem of Thoma pipette and fill the counting chamber (Neubauer (or 'Improved Neubauer')) with the diluted blood (0.1 ml of diluted blood in each square).
- 6. Leave on bench for 2-5 minutes for the cells to settle.
- Count the RBCs under the microscope using the (x40) objective lens and lowering the condenser. The RBCs are counted in 1/5th of the RBC square i.e. 0.2 mm<sup>2</sup>

**RBC Counting Area**: The large center square is used for RBC counts. This area is subdivided into 25 medium squares, which in turn are each divided into 16 squares. Of the 25 medium squares, only the four corner squares and the center square within the large center square are used to perform RBC counts.





areas of the grid where RBC are counted

Area of median square =  $0.2 \times 0.2 = 0.04 \text{ mm}^2$ 

Volume of median square =  $0.2 \times 0.2 \times 0.1 = 0.004 \text{ mm3}$ 

Volume of 5 median square =  $0.004 \text{ x} 5 = 0.02 \text{ mm}^3$ 

### Calculations

N = the number of cells in 0.02 ml of diluted blood (in 5 squares)

RBC count  $\mu l = \frac{No. of RBC \times Df}{counted volume}$ RBC count  $\mu l = \frac{No. of RBC \times 200}{0.02}$ 

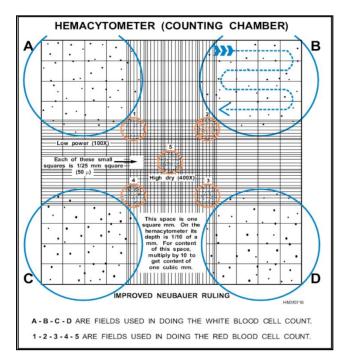
RBC count  $\mu$ l = *No. of RBC x* 10000

### **Normal Ranges**

- Men:  $5 \pm 0.5 \times 10^{12} / L$
- Women:  $4.3 \pm 0.5 \times 10^{12}/L$

### **Automated Red Cell Count**

Red cells and other blood cells can be counted in systems based on either aperture impedance or lightscattering technology. Because large numbers of cells can be counted rapidly, there is a high level of precision. Consequently, electronic counts have rendered the RBC of much greater clinical relevance than was possible when only slow and imprecise manual RBC count was available.



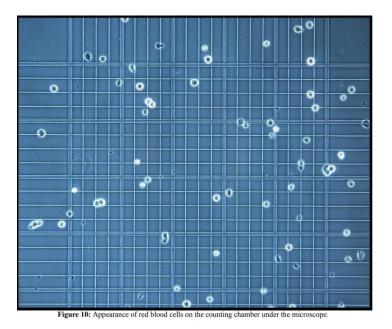
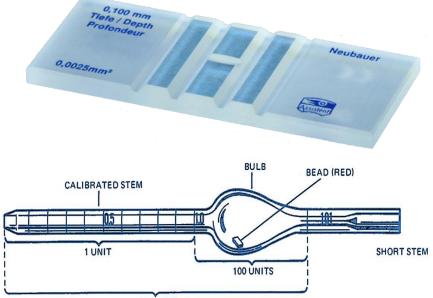


Figure 9: Illustration showing different parts of the counting chamber.



101 UNITS

