WBC manual count using hemocytometer

Prepared by

Hadeel Al Sadoun

Objectives

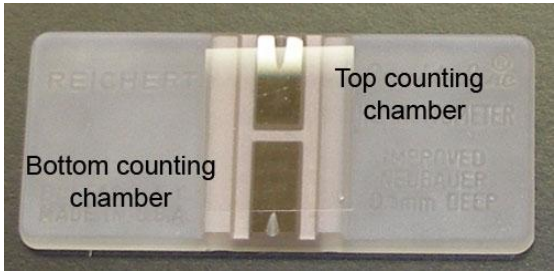
- To accurately count WBC in Chamber.
  - To perform reliable dilution of blood cells
  - To calculate the number of cells/µL

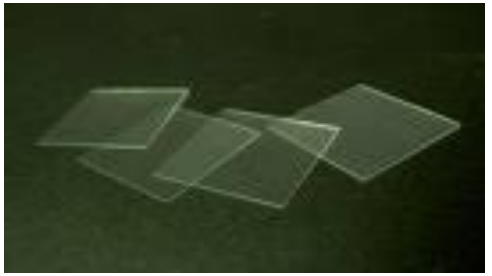
Principle

- Whole blood collected in EDTA is diluted according to the type of cell count obtained.
- The diluted blood suspension is then placed in a chamber and the cell counted
- The count is multiplied by dilution factor and reported as number of cells per microlitter (µL) of whole blood

Material

- Hemocytometer with Neubauer grid.
- Cover glass
- Diluents
- Microscope.





Methodology

- Put the cover slip or glass slip on the top of grid area in the Chamber (use air tight technique)
- Dilute you sample:
  - 1: 20 for WBC count
  - 1:200 for RBC count and platelets
- Load your sample into the laoding area in the chamber
- Count the cells in the 4 large squares for WBC
- calculate the number of cells counted / µL

Sample dilution

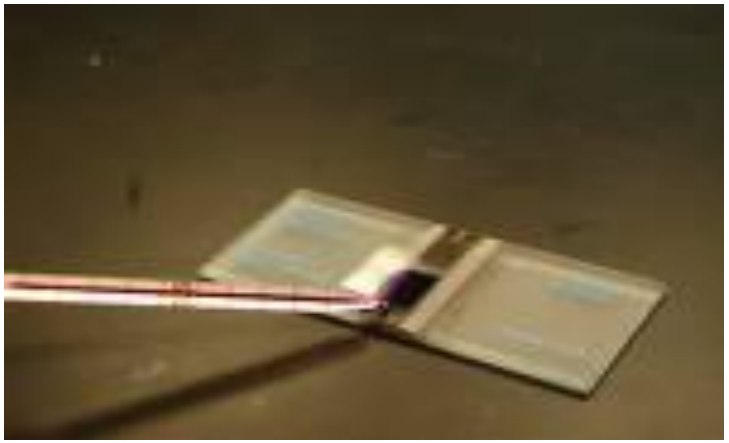
- Dilution of whole blood sample:
  - Diluents:
    - Acetic acid (CH_3COOH)
    - Or : dis. H_2O
  - Purpose:
  - Dilute the amount of WBC , RBC to be able to count it. (NR RBC: M 4.3-6.2 x 10^6 /µL) (F: 3.8-5.5 x 10^6 /µL)
    (NR WBC: 4.3-10.8 x 10^3/µL)
  - To lyses the RBC and platelets (the diluents lyses also the WBC but takes longer time) (time factor is critical)

Methodology

- Dilution:
  - 1:20 dilution or 1:50 (ex: chronic lukemia)
  - (1+19=20)
  - (50µL of blood + 950 µL diluent)

- Loading the sample:

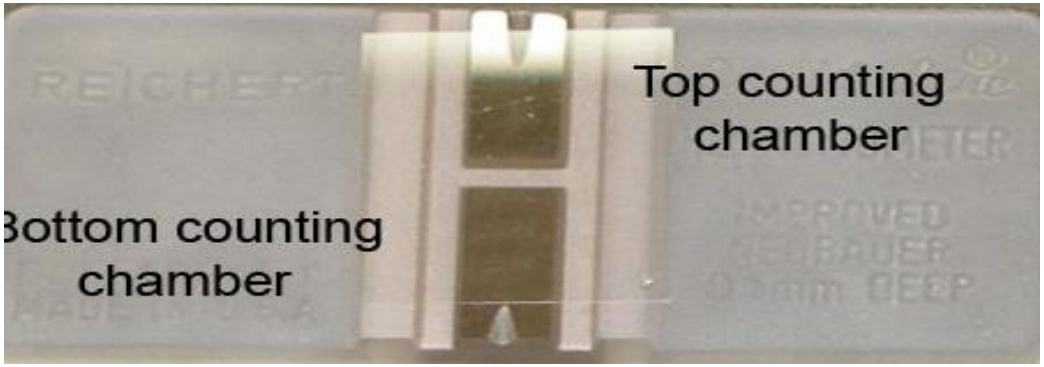
Hemacytometer loading.lnk



WBC count

The hemocytometer contains 2

Neubauer counting chamber →



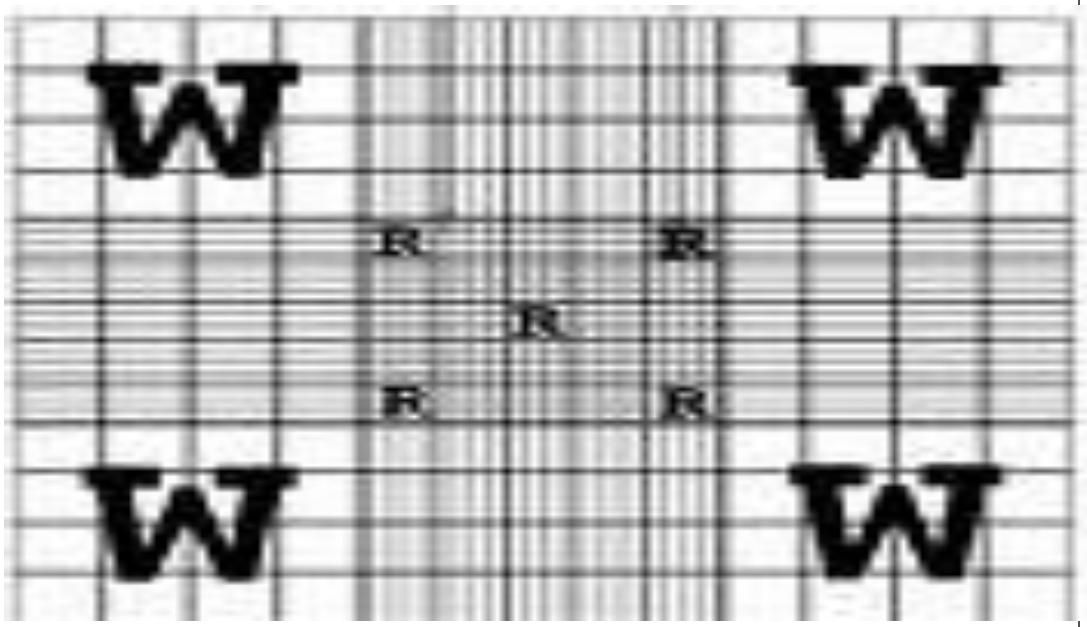
Each chamber contains:

***4 WBC counting squares**

***Each contains 16 squares**

100 RBC= 10 Platelets= 1 WBC

Chose 90° lines, count only the cells that on those lines (ex: L-shape)
apply it to all squares for maximum accuracy



Calculation

- Cells/ μL =
- $$\frac{\text{no. of cells in 1 large square x Dilution factor}}{\text{volume factor (0.1)}}$$

Dilution factor= reciprocal of dilution (20)

Volume factor = (width x length x height)= 0.1