WBC manual count using hemocytometer

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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 your sample:
  - 1:20 for WBC count
  - 1:200 for RBC count and platelets
- Load your sample into the loading 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$_3$COOH)
    • Or : dis. H$_2$O
  • 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 leukemia)
  - $(1+19=20)$
  - $(50\mu\text{L of blood} + 950 \mu\text{L diluent})$

- **Loading the sample:**
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 =
- no. of cells in 1 large square x Dilution factor

\[
\text{volume factor (0.1)}
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Dilution factor = reciprocal of dilution (20)
Volume factor = (width x length x height) = 0.1