

Laboratory -2-

The Enumeration of White Blood Cells (Total Leucocyte Count)

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A WBC count is a test to measure the number of white blood cells (WBCs) in the blood. The normal number of WBCs in the blood is 4,000-11,000 white blood cells per microliter μl or mm^3 .

An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia.

Higher values – leukocytosis: – physiological: after effort, after meals, women: menstruation, pregnancy, childbed – pathological: infection, inflammation, poisoning

Lower values – leukopenia: anaphylactic shock, viral infections, X-ray exposure.

WBCs have nuclei and all cellular organelles and are classified as either granular or a granular, depending on whether they contain cytoplasmic granules that can be visualized by staining.




Granular leukocytes include:

Depending on the type of dyes staining their granules. Their nuclei have lobes.

1- Neutrophils(60-70% of WBCs)

2- Eosinophils(2-4% of WBCs)


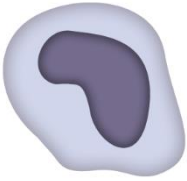
3- Basophils (0.5-1% of WBCs)

Cell type	Image	Lobation
Neutrophil		Multilobed, i.e. having more than two lobes. Fine, faintly pink.
Eosinophil		bi-lobed, i.e. having two lobes Full of pink-orange
Basophil		bi- or tri-lobed Large blue

A Granular leukocytes include

Lymphocytes (20-25% of WBCs)

Monocytes (3-8% of WBCs)

Lymphocyte		Deeply staining, eccentric
Monocyte		The nucleus is Kidney shaped

Principle:

Blood sample is mixed and diluted with Turk's Solution which will lyse red blood cells, and will darken WBCs to facilitate counting by the hemacytometer.

Apparatus and Reagents

- 1- Neubauers chamber with coverslip or hemacytometer
- 2- A white blood cell pipette
- 3- Microscope
- 4- Diluting fluid (Turk`s solution).
- 5- Whole blood.
- 6- Lancet
- 7- Alcohol 70%

Turk`s solution

- 1- Glacial acetic acid (lyses red blood cell)
- 2- Gention violet (1% aqueous solution) :- stain white blood cell
- 3- Distilled water

Procedure

- 1- Clean the hemocytometer and its cover slip with an alcohol pad then dry it.

- 2- Draw blood in a clean dry WBC pipette up to the mark 0.5 with all possible accuracy.
- 3- Draw the diluting up to mark 11.
- 4- Mix the contents of pipette for minutes.
- 5- Dispel the first 4 drops of the contents.
- 6- Adjust the Neubauers chamber. It must be clean and dry. By holding the cover slip between the fingers at the edges, place it in such manner that both the ruled platforms are evenly covered by it. Load it with the mixture, by holding the pipette at angle of 45° degree and touching the space between the cover slip and the chamber by the point of the pipette, an appropriate drop of the mixture is allowed to run under the cover slip by capillary action.
- 7- Allow 2 minute for setting of cells then count.

Diluting pipettes: - are capillary (very thin) pipettes with a mixing chamber. In the mixing chamber there is a mixing bead, colored in red for the red blood cell pipette and in white for the white blood cell pipette. On the capillary part of the pipette are marks for 0.5 and 1 μ l, and above the mixing chamber is a mark for 101 μ l on the red cell pipette and for 11 μ l on the white cell pipette. A rubber tube with a mouthpiece is attached to the top-end of the pipette.

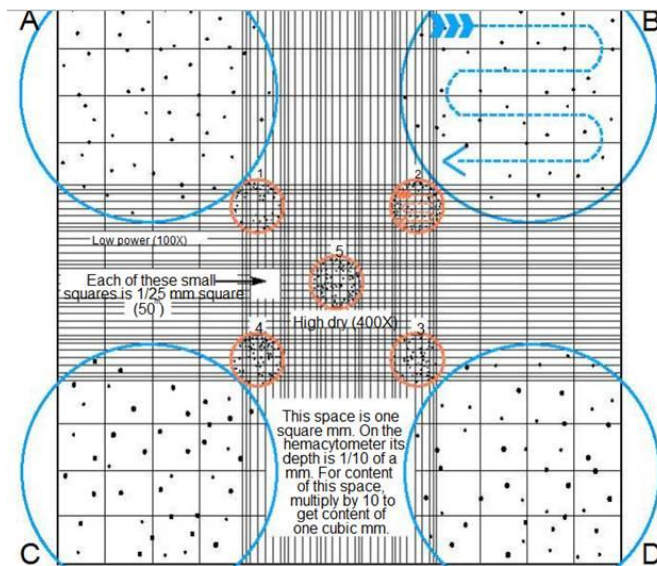
Calculation

Each large square has an area of 1 sq. mm². the depth of the chamber is 1/10 mm. the volume of fluid covering each large squares is 1mm²*1/10 mm = 1/10 mm³. Then the number of cells in 1 cm of diluted blood will be (10 × M). As the blood diluted 20 times. So

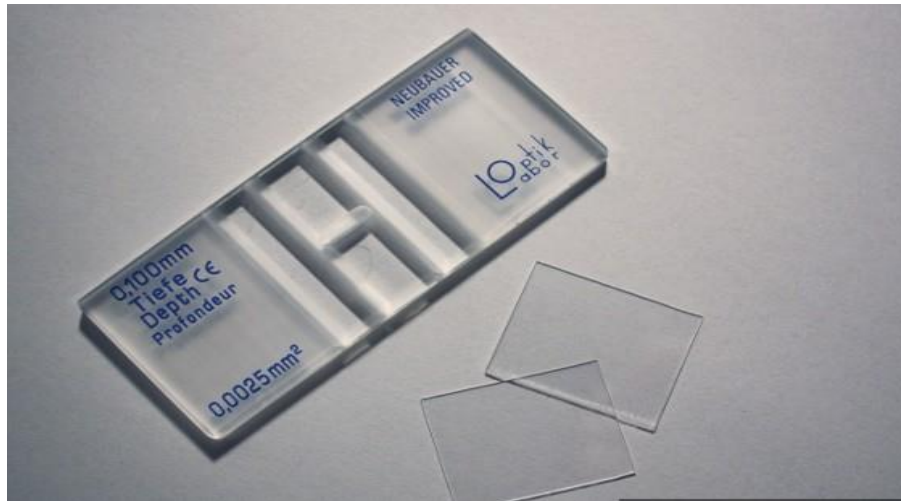
Final equation of white blood cell count = N × 200



Diluting pipettes



Neubauers chamber with coverslip or hemacytometer under microscope



Neubauers chamber with coverslip or hemacytometer

Source of Error:

1. Sampling error in collection of blood.
2. Equipment error in the pipette and chamber.
3. Technical errors involved in the exercise from the pipette to the final count.
4. Inherent of field errors of the distribution of cells in the chamber. This can be minimized by counting large number of cells.

Normal Value

Infant's	15,000-20,000 cell/mm ³
Children	4,500-13,500 cell/mm ³
Adult	4,000-11,000 cell/mm ³

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