The Total leukocyte count (WBCs count)

White blood cells are a part of body's immune defense system, white blood cells count is important in the diagnosis of disease especially when accompanied by a differential white cells count. Leukocytes are found throughout the body, including the blood and lymphatic system

Clinical Applications

Leukocytosis is the increase in total WBC count (WBCs count is in excess of 11,000 cells/mm3), which occurs in both physiological and pathological conditions such as Pregnancy, Following exercise, Emotional stress, Food intake, Allergy , Common cold , Bacterial infection such as and urinary tract infection and tonsillitis , Hemolytic disease of new born, Tuberculosis , Glandular fever., Leukemia,.

Leukopenia is the decrease in total WBC count (WBCs count is below 4,000 cells/mm3), This condition is indicative of depressed bone marrow or a neoplasm of the bone marrow, the term which is generally used for pathological conditions only. It occurs in the following pathological conditions such as Viral infections as infectious hepatitis and some bacterial infections such as typhoid fever, brucellosis, Rheumatoid arthritis, Systemic Lupus Erythematosis, certain drugs such as radio therapy and chemotherapy

Normal Value

Infants	15,000-20,000 cell/mm3
Children	4,500-13,500 cell/mm3
Adult	4,000-11,000 cell/mm3

The principle :- The blood is diluted 20 times with the WBCs diluting fluid , the leukocytes are then counted in 4 corners squares of the improved Neubauer chamber.

Purpose :-the WBCs count is determine the number of circulating WBCs in the body

Material and method

1-Hemocytometer apparatus composed of

a- counting chamber (Neubauer,s chamber)

b- WBCs counting pipette.

c- Rubber tube with a plastic mouth piece for drawing the fluid into a pipette.

d-Special hemocytometer coverslip

2-Diluting fluid.

3-Microscope.

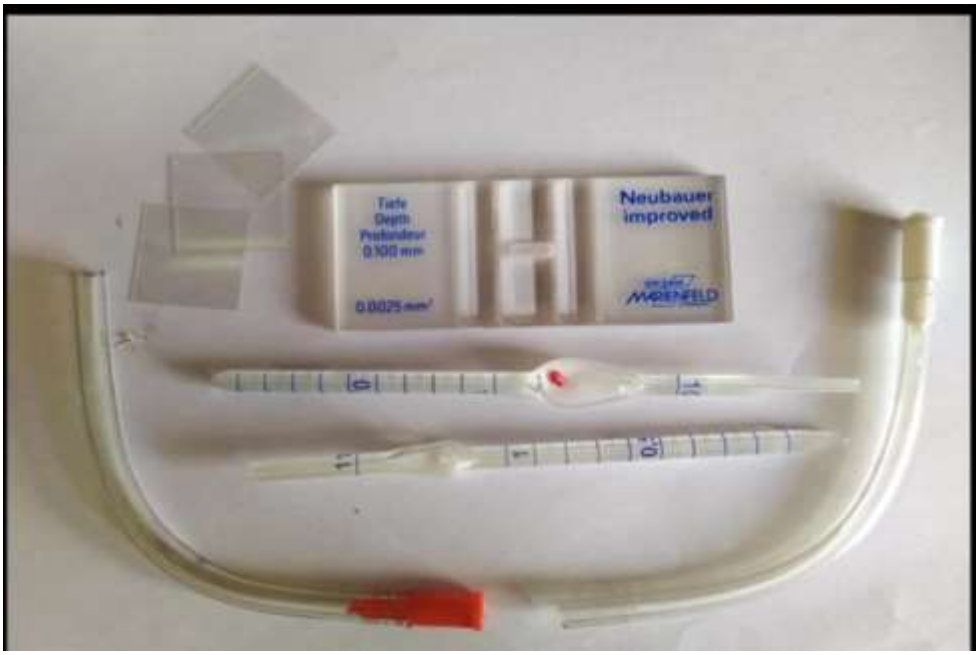

Figure (1): Hemocytometer apparatus

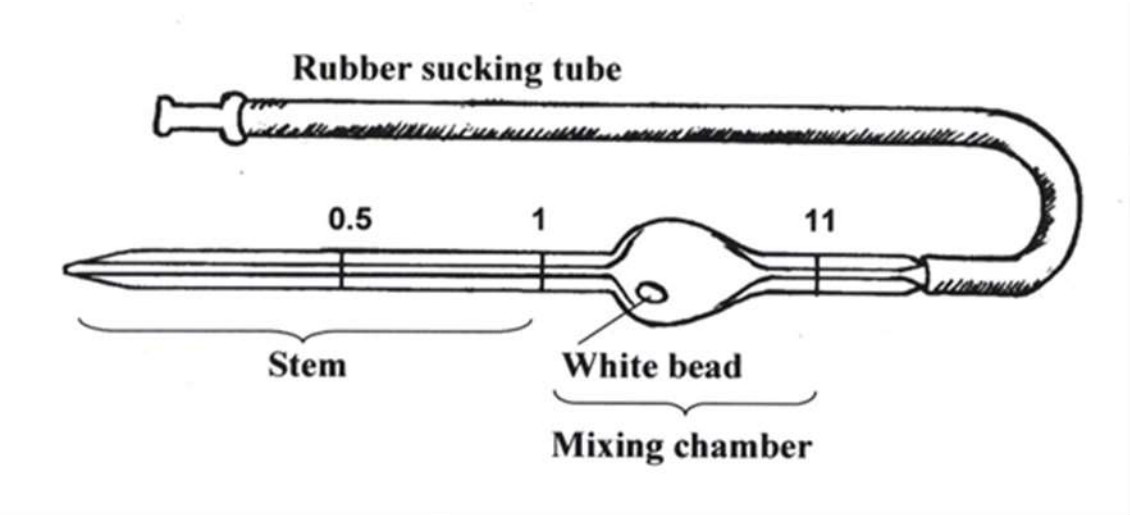

Figure (2): WBCs counting pipette

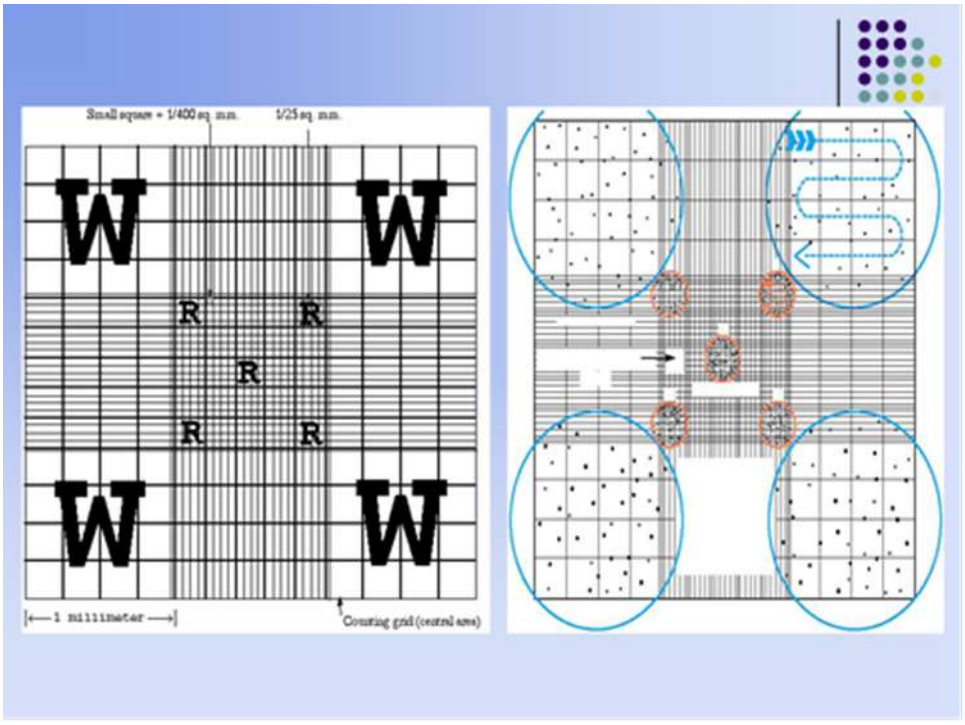

Figure (4): WBCs counting procedure

Diluting fluid

Turk's solution used for diluting WBCs, this diluting fluid contains an acid solution that lyses the RBCs and a stain that stains the nuclei of the WBCs and allows for easy identification and counting; and an appropriate is:

Distilled water (solvent) 98 ml

Glacial acetic acid (hemolysis of RBC) 1.5 ml

Crystal gentian violet (1% solution) 1 ml

(stains WBC nuclei**)**

Procedure

1.Examine the chamber under the low power objective of microscope, without the coverslip, in order to understand the ruling .

2.Clean the counting chamber and the coverslip, place the chamber on a flat surface ,and then put the coverslip on the surface of the chamber.

3.Obtain a fresh whole blood sample (usually EDTA anticoagulant), but the sample can be capillary blood from a finger puncture.

4.Draw blood in a clean dry WBC pipette up to the mark 0.5. Make sure that there are no air-bubbles .

5.Wipe the outside of the pipette tip clean to remove excess blood .

6.Draw the diluting fluid up to mark 11 (dilution 1:20).

7.Withdraw the pipette from the diluting fluid ,and wipe the outside with a piece of clean gauze .Close the tip of the pipette with the thumb, remove the sucker, place the middle finger over the top and mix well by shaking for 2 minute

8.Discard the first 2 or 3 drops from the pipette to allow the dilution in the glass bulb to reach the tip end of the pipette .

9.Fill the chamber by holding the pipette at an angle of 45° and lightly touching the tip against the edge of the coverslip; allow the diluted blood to flow evenly and slowly under the coverslip by capillary action. Overfilling or underfilling the chamber will result in erroneous counts .If this occur , the chamber should be cleaned and refilled .

10.Leave the chamber for 2 minute ,for the white cells to settle down, and place it on the microscope stage .

11-Cells are scanned under a 10x objective to determine the distribution.

12-Use the 40x objective to count WBCs in each of the four of the corner secondary square on both sides of the chamber

Calculation:- No. of cells in large corner square = N.

$$\text{No. of WBCs/mm3} = \text{Cells counted} \times \frac{\text{Dilution factor}}{\text{Volume factor}} = NX\ 20/0.1=NX200$$ (In one square)

$$\text{No. of WBCs/mm3} = N \times \frac{20}{0.4} = N \times 50$$

(In four squares)