Total White Blood Cells Counting

The WBC count is a basic procedure. White cells are still counted manually in smaller laboratories. Although generally replaced by automated cell counts, manual counts are still done in certain circumstances and are the usual method of counting cells in other body fluids, such as cerebrospinal fluid and synovial fluid.

Normal Value = 4000 – 11000 cells/1mm^3 of blood

Important Terms:

- **Leukocytosis:** An increase in the WBC count above the normal upper limit and may occur in many acute infections, especially bacterial infection, in sever malaria, after hemorrhage, during pregnancy, postoperatively, in some forms of anemia, in some carcinomas and leukemia.
- **Leukopenia:**A decrease below the normal lower limits and may occur with certain viral infection, typhoid fever, after radiation therapy, after the administration of certain drugs and in pernicious anemia.
- **Leukemia:**is characterized by uncontrolled proliferation of one or more of the various hematopoietic cells and is associated with many changes in the circulating cells of the blood.
  There are 2 types of Leukemia depending on the predominant type of WBCs seen:
  1. Lymphocytic Leukemia
  2. MyelocyticLeukemi

- Leukemia can be further subdivided into acute and chronic. In the acute form, the disease progresses rapidly and there is a marked morphological changes, while in the chronic form, the changes are not rapid and not marked.
- A child´s leukocyte count usually shows a higher variation during a disease than an adult´s count.
- There is also an increase in the WBC count after a strenuous exercise, emotional stress, and anxiety.

Objective of the Experiment:

1. The presence of infection and follow the progress of certain diseases
2. Presence of Leukemia
3. Monitor the body response to various treatments
4. Conditions or diseases that may weaken the immune system, like HIV infection, chemotherapy, radiation therapy causing a drop in WBC count.
5. Monitor bone marrow function

Principle of the Experiment:

The method depends on accurate dilution of a measured volume of a blood using a special type of dilution fluid, known as Turk´s solution.

Apparatus and Reagents Required:

1. Special WBC pipette
2. Counting chamber (also called hemocytometer). The improved Neubauer chamberhas 2 types of counting squares, one for leukocytes counting which is located at the corners of the chamber and the other one for RBCs counting, which is located in the center of the chamber.
3. Cover slip

4. **Dilution colored fluid: (Turk's solution)** which is prepared by using 1 ml of glacial acetic acid, 1 ml of diluted solution of gentian violet or methylene blue and then complete the volume to 100 ml with distilled water. Glacial acetic acid is used for the lysis of the RBCs, while the gentian violet is to stain the WBCs.

Procedure:

- Obtain a WBC pipette and a hemocytometer. Place the hemocytometer on the stage of the microscope and examine so you can identify the counting area. Use the low power to find the central $1mm^3$ square and the corners $1mm^3$squares
- Clean and dry the pipette before and immediately after using it with distilled water
- Clean the hemocytometer with DW and dry it with a soft tissue. Do not use an organic solvent such as alcohol, acetone or others because it will damage the hemocytometer counting area
- Place a few milliliters of Turk's solution in a clean watch glass
- Clean the tip of your finger with 70% alcohol. Let it dry and then lance the finger to obtain a drop of blood. Wipe off the first drop with a tissue and allow a second drop to collect
- Place the mouth piece of the WBC pipette between your lips keeping the pipette in a horizontal position. Insert the tip of WBC pipette into the drop of blood. Suck gently on the rubber tube to draw blood up to exactly at the 0.5 mark on the WBC pipette. Be sure that the pipette tip is inside the blood. If air bubbles enter the pipette, blow them out and repeat.
- After drawing blood to 0.5 mark of the pipette, immediately draw the Turk's solution from the watch glass while still holding the pipette in a horizontal position till you reach the 11 mark on the pipette.
- Then withdraw the pipette from the solution and start rotating the pipette between your fingers for 5 minutes while holding it in a horizontal position to avoid spilling the contents of the pipette.

- At the end of the 5 min, discard 3 drops of the mixture and then prepare the hemocytometer and the cover slip on top of the area where the counting chambers are located.
- Now on the laboratory bench, place a drop of the mixture at the edge between the hemocytometer slide and the cover slip and let the drop to spread through the capillary movement through the space between the cover slip and slide. Let it stand for 1 min in order for the cells to settle down.
- Then put the slide under the microscope and examine and count the WBCs at high power magnification.

Calculations:

1. **From the WBC pipette,**

0.5 unit of blood is diluted to 11 units with Turk's solution

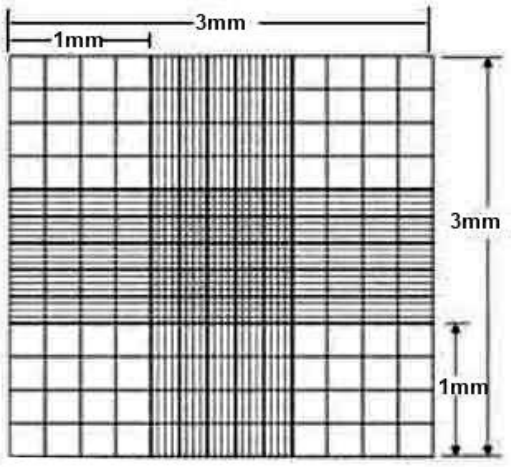
0.5 unit (B) → 11 unit (B+T)

Mix by rotating the pipette for 2 – 5 min then discards 3 drops which are equivalent to 1 unit.

0.5 unit (B) → 11 unit (B+T) – 1 unit (B+T)
0.5 unit (B) → 10 unit (B+T)
0.5 unit (B) ≡ 10 unit (B+T)
1 unit (B) ≡ 20 unit (B+T)
1 mm3 (B) ≡ 20 mm3 (B+T)
1/20 mm3 (B) ≡ 1 mm3 (B+T)

1 mm3 (B+T) ≡ 1/20 mm3 (B)

2. From the hemocytometer slide,



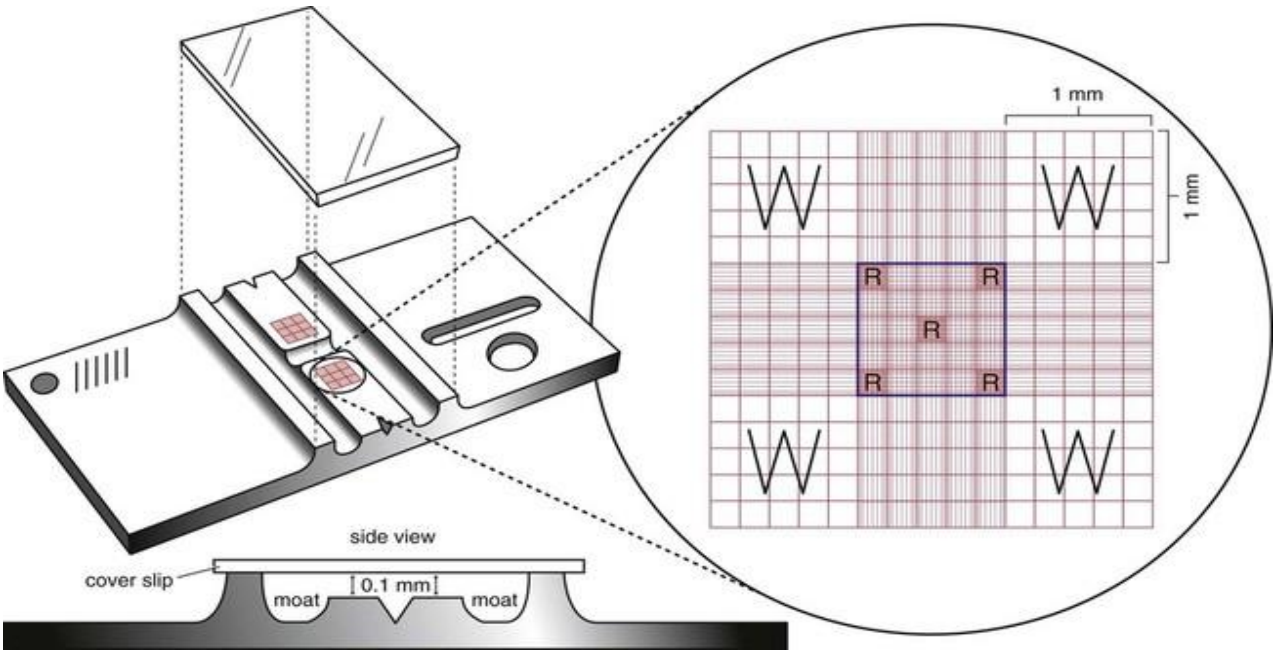
Calculate the volume of 1 corner square

V = L * W * D
= 1mm * 1mm * 0.1mm
= 0.1 mm3 (B+T)

Volume Cells
0.1 mm3 (B+T) W
1 mm3 (B+T) ?
Then 1 mm3 (B+T) = 10W
Since 1mm3 (B+T) ≡ 1/20 mm3 (B) ---------- (from the pipette equation)
Then:

1/20 mm3 (B) 10W
1 mm3 (B) ?

1 mm3 (B) = 200W



Hemocytometer slide (improved Neubuer)