**EXPERIMENT NO.: 8** **DATE:**

**AIM: TO ESTIMATION TOTAL WBC (LEUCOCYTE) COUNT OF OWN BLOOD SAMPLE**

**REQUIREMENTS:**

Microscope, Haemocytometer, Thomas coverslip, WBC diluting fluid, Cotton swab, Pricking Needle/Lancet, Napkin, Plastic Sheet

**PRINCIPLE:**

The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid (water: glacial acetic acid: gentian violet = 97:2:1) and the cells are counted under low power of the microscope (10X) by using a counting chamber. The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leukocytes to locate the WBC under microscope.

**THEORY:**

- White blood cells, present in plasma take part in body defense against invading micro-organisms.
- They are produced from the pluripotent stem cell in the bone marrow in adults. In case of foetus haemopoiesis occurs in liver and spleen.

**Clinical Significances of total leukocyte count:**

- Increase in total leukocyte count of more than 10,000/cu mm (µl) is known as leukocytosis and decrease of less than 4 000 cu mm (µl) as leukopenia.

**Causes of leukocytosis:**

- It is common for a transient period in infections (bacterial, protozoal (malaria), or parasitic),
- Leukocytosis is also observed in severe hemorrhage and in leukemia ii. High temperature
- Severe pain iv. Accidental brain damage.

**Causes of Leucopenia:**

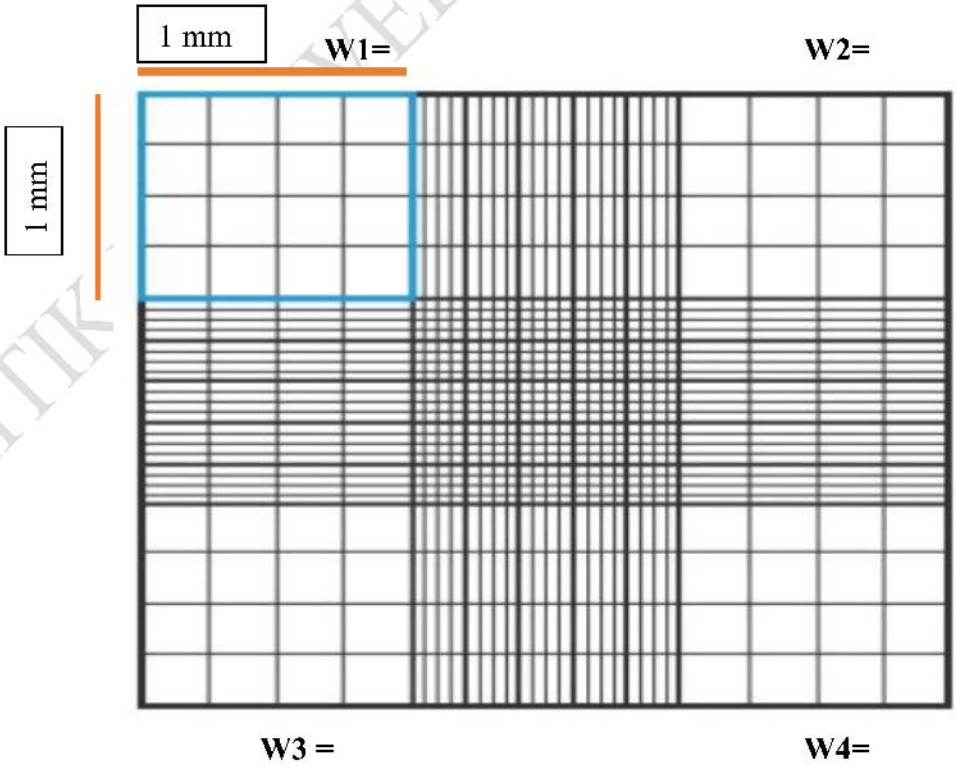
- Certain viral (hepatitis, influenza, measles, etc.), and bacterial (typhoid, paratyphoid, tuberculosis, etc) infections
- Primary bone marrow depression (aplastic anaemia)
- Secondary bone marrow depression (due to drugs, radiation, etc.) Iv. Anaemia (iron deficiency megaloblastic etc).

**NORMAL RANGES:**

About 4,000to 10,000 per µl or cubic mm of blood

**PROCEDURE:**

- Clean microscope, neubauer counting chamber and Thomas cover slip,
- Place the Thomas cover lip on Neubauer counting chamber and adjust the Neubauer counting chamber under low power objective lens i. e 10X.
- Make ready neat and cleaned WBC pipette to collect blood from the ring finger
- Sterilize the ring finger with 70% of alcohol and pricked boldly with the help of pricking needle.
- 1st drop discarded, then hold the WBC pipette slightly down position like tip of the Pipette touch the pricking site.
- Take the WBC pipette tube of the next end in the mouth and try to pull blood in capillary without AIR bubble till the making of 0.5.
- Then pill WBC dilution fluid upto the mark 11.
- Make a 1:20 Dilution.
- Give a node to the WBC pipette tube and mix the fluid gently for 1-2 minutes.
- Open the node of WBC Pipette and place the tip of WBC pipette like that the fluid portion enter between the gap of Thomas cover slip and Neubauer counting chamber.
- Allowed the fluid to spread on the counting.
- Try to count WBC as shown in below figure.



**CALCULATIONS:**

- Total Number of white blood cells in 16 small square[ 64 smallest square] = N

  N means = W1+W2+W3+W4 =
- Length (L) of 1 small WBC square = 1mm
- Width (W) of 1 small WBC square= 1mm

  So area of 1 small square (L X W) = 1 mm X 1 mm= 1 $mm^2$ or 1 sq. mm
- Height (Thickness) of the counting chamber 1/10 mm = 0.1 mm
- Volume of fluid in 1 small square (L X W X H ) = 1 mm X 1 mm X 0.1 mm = 0.1 $mm^3$
- So, Volume of fluid in 4 small square = 0.1 X 4 = 0.4 cmm or 0.4 $mm^3$

  If,

  0.4 cmm or (4/10) cmm, of diluted fluid contains N WBC

  So, 4 cmm (4 X 1 cmm) of diluted fluid contains = N X 10 WBC

  1 cmm of diluted fluid contains = N/4 X 10 WBC
- Dilution factor is 0.5 in 10 or 1 in 20

  Therefore total number of WBC in undiluted fluid = N/4 X 10 X 20

  = N X 50 /cmm

  =

  =

**RESULT: Total WBC count of my own blood is _____/cmms**

**(Normal WBC count 4000 to 11,000/cmm of blood)**

**CONCLUSION:** Therefore, my blood is in **normal/ abnormal**

**CLINICAL SIGNIFICANCES OF DIFFERENTIAL LEUKOCYTE COUNT:**

| Types of WBC, elevated in plasma | Significances |
|---|---|
| Neutrophils | Acute bacterial infections, hemorrhage, diabetic acidosis |
| Basophils | Increase in types of blood dyscrasias |
| Eosinophils | Increase in parasitic and allergic conditions, pernicious anemia |
| Monocyte | Hodgkin's disease, lipid storage disease, recovery from severe infections, monocytic leukemia |
| Lymphocyte | Viral and chronic bacterial infections, acute and chronic lymphocytic leukemia, antigen reaction |

**Increased WBC Count (Leukocytosis):**

The count is more than 11,000/cmm.

1. Infections mostly acute bacterial give rise to an increase in the WBC count.
2. Trauma and stress.
3. Hemorrhage.
4. Dehydration.
5. Steroid therapy.
6. Inflammations.
7. Thyroid hormone increases.
8. Leukaemias or another myeloproliferative process.
9. Other malignancies may increase WBC count.

**Decreased WBC Count (Leukopenia):**

The count is less than 4000/cmm.

1. Drug toxicity causing bone marrow depression.
2. Cytotoxic drugs.
3. Bone marrow failure.
4. Severe infections.
5. Bone marrow infiltration by the tumors or myelofibrosis.
6. Dietary deficiency like vitamin B12 and iron deficiency.
7. Hypersplenism.
8. Autoimmune diseases.
9. Low TLC may be seen in typhoid fever.



**SIGNATURE OF TEACHER**