S. S. College, Jehanabad

Department: Zoology

Class: M.Sc. Semester I

Subject: Zoology

Topic: Enumeration of WBC – Total Leukocyte Count (TLC)

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ENUMERATION OF WBC - TOTAL LEUKOCYTE COUNT (TLC)

White blood cell is a type of cells and an important component of blood that lacks hemoglobin, but has every cellular organelle, and defends the body against the foreign invasion or non-self. It is capable of motility and is also called as **leukocyte** or **white corpuscle**. The white blood cells are largely involved in ingesting foreign materials and cellular debris by acting as scavenger, in destroying infectious agents and cancer cells, or producing antibodies upon interaction with foreign antigens or infectious agents. They are larger than the RBC in size and also differ in the shape; white blood cells (WBCs) are rounded, amoeboid and irregular in shape while red blood cells (RBCs) are biconcave discoid. The size of WBCs is about 15µm while that of RBCs is 7.5µm.

Differences between Red Blood Cells (RBCs) and White Blood Cells (WBCs)

S.N.	**Difference**	**Red Blood Cells (RBCs)**	**White Blood Cells (WBCs)**
1.	**Other name**	Also called "Erythrocytes"	Also called "Leukocytes"
2.	**Origin**	They are produced in red bone marrow.	Mostly bone marrow, also produced in lymph nodes, spleen, etc.
3.	**Nucleus**	Nucleus Absent	Nucleus Present
4.	**Size**	Smaller than WBCs, 7.5µm	Larger than RBCs, 15µm
5.	**Color**	Filled with hemoglobin (Red)	Colorless, No Pigment
6.	**Production**	2 million RBCs per second	Fewer WBC than RBCs
7.	**Life Span**	RBCs have an average lifespan of 120 days	WBCs live anywhere from a few days (5-21 days)
8.	**Number**	5 million RBCs in every cubic mm of blood	3,000 – 7,000 WBCs in every cubic mm of blood
9.	**Number Increments**	Number increases during exercises and high altitudes	Number increases during infection
10.	**Process of Formation**	Formation of RBC is called "Erythropoiesis"	Formation of WBS is called "Leucopoiesis"
11.	**Shape**	Circular, Biconcave	Rounded and Amoeboid, Irregular

12.	**Motility**	Non-Motile	Generally Motile
13.	**Movement**	Doesn't leaves Blood Vessels	Come out of blood Capillaries
14.	**Rouleaux Formation**	Form stacks called Rouleaux	Rouleaux formation absent
15.	**Types**	One Type	Five Types
16.	**Circulatory system**	Cardiovascular system	Cardiovascular and lymphatic systems.
17.	**Function**	Transport of Respiratory Gases (Oxygen and Carbon Dioxide)	Defense Mechanisms
https://microbiologyinfo.com/differences-between-red-blood-cells-rbc-and-white-blood-cells-wbc/			

A healthy adult human has between 4,500 and 11,000 WBCs per cubic millimeter of blood (5,000 – 10,000 in men & children, while 4,500 – 11,000 in women). The number of WBCs is not constant; it depends on various physiological conditions of the body. It fluctuates even in the day; resting period reflects lower number of WBCs, while intense physical activities, exertion and exercise results in the increase in the number of WBCs. However this fluctuation is normal and occurs in range. The deviation from the range is indicative of some pathological condition, like an abnormal increase in WBCs, i.e. **leukocytosis** can be observed in the case of convulsions, acute emotional reactions, pain, pregnancy, labor, infections, intoxication and certain cancers. On contrary, certain types of infections, such as viral infection, chronic anemia, malnutrition, and anaphylaxis (Greek ana-, meaning "against", and phylaxis, meaning "protection"; a severe, potentially life-threatening allergic reaction) result in abnormal decrease in the number of WBCs, a condition known as **leukopenia**. However certain drugs are also known to decrease the number of WBCs in the individual.

Principle

WBC diluting fluid is used for performing the WBC (Leucocyte) count. Glacial acetic acid lyses the red cells. Gentian violet slightly stains the nuclei of the leucocytes. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid and the cells are counted under low power of the microscope by using a counting chamber. The number of cells in undiluted blood is reported per cumm (µl) of whole blood.

Equipment

1. Pipettes – Thoma peipette for WBCs or micropipette – 20µl is the desired volume.
2. Improved Neubauer's chamber with cover slips.
3. Light microscope.

4. Clean gauze

Reagents and solution

1. Diluting fluid or dilution buffer – Procured readymade or can be prepared as Türk's solution, such as from HiMedia, Mumbai, India.
2. 70% ethyl alcohol for cleaning microscope lens

   Türk's solution: For WBC count, generally Türk's solution is used. Türk's solution is a hematological stain (crystal violet or aqueous methylene blue) that lyses the membrane of RBCs, WBCs and platelets within a blood sample, and stains the nuclei of the WBCs allowing easy identification and counting. This solution contains acetic acid that lyses the membrane. Composition of Turk's solution is following:

   Glacial acetic acid *1ml*

   Gentian violet *2ml (1% aqueous solution)*

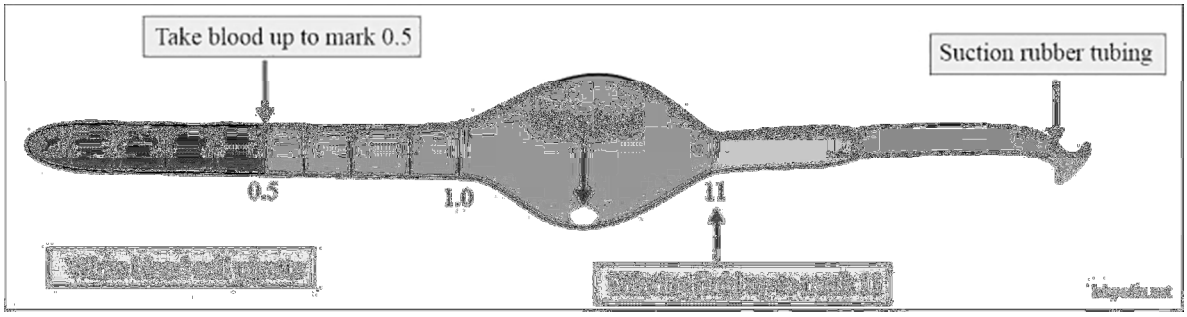
   Distilled water *100 ml Türk's solution*

   Preparation of Türk's solution:

   - Add 2 ml of glacial acid (2 + 1), 1 mL gentian violet (1 %), and add 97 ml of distilled water to make up to 100 ml solution under fume hood.
   - Add Gentian violet until the pale blue-violet colour is developed.

Procedure

1. Clean the hemocytometer and its cover slip with 70% ethyl alcohol and then dry with a dry wipe.
2. Draw blood in a clean dry WBC pipette up to the mark 0.5 with all possible accuracy.
3. Draw the diluting fluid up to mark 11 (dilution 1 in 10).

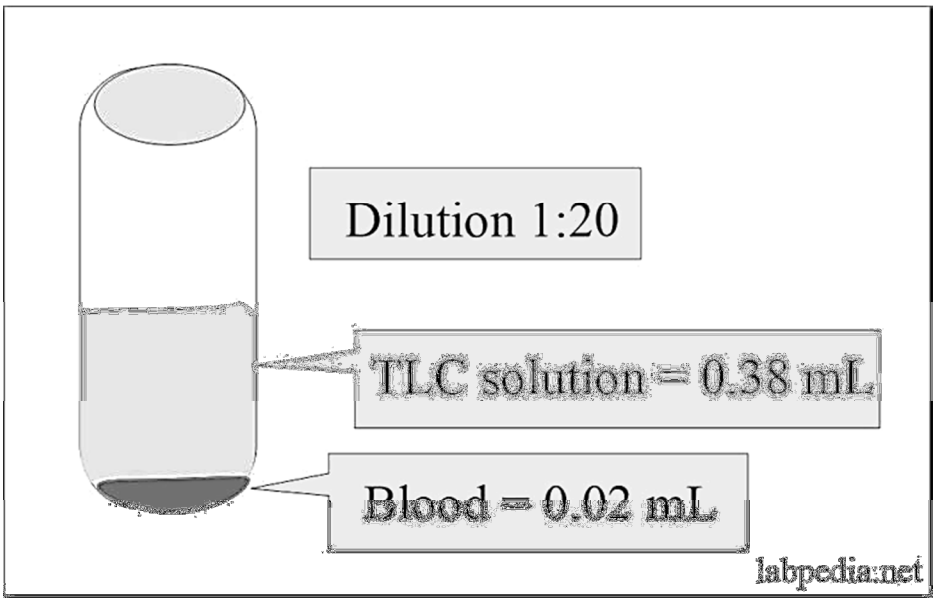


   For tube method, take 0.02 ml blood and mix it with diluting fluid 0.38 ml of which is taken in a small tube. Mix the blood with diluting fluid (1:20 dilution).

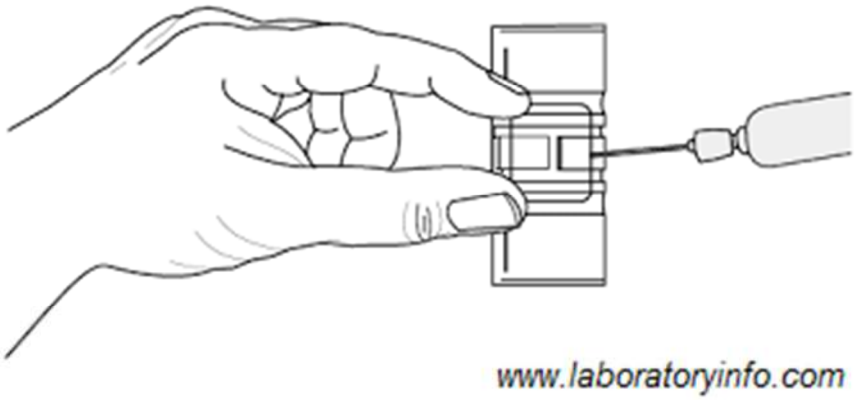
   Tube method is said to be more accurate in comparison to Thoma's pipette.

4. Mix the contents of pipette for minutes.

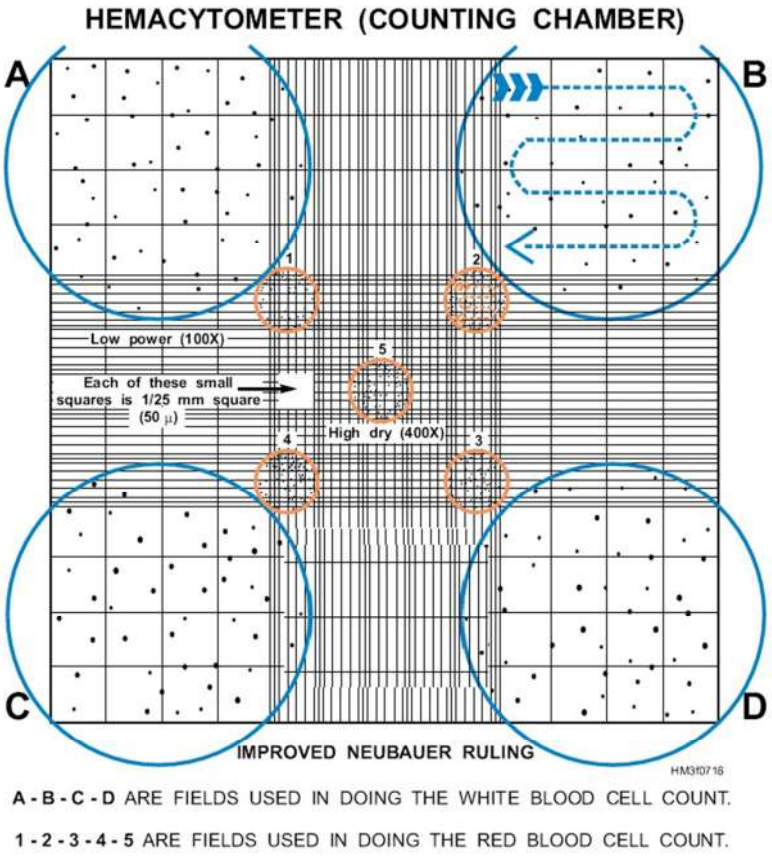
5. Dispel the first 4 drops of the contents.



6. Adjust the clean and dry Neubauers chamber. By holding the cover slip between the fingers at the edges, place the cover slip in such manner that both the ruled platforms are evenly covered. Now, load the Neubauers chamber with the pipette contents by holding the pipette at angle of 54 degree and touching the space between the cover slip and the chamber through pipette mouth. Neither too much nor meager, an appropriate drop of the content is allowed to run under the cover slip by capillary action.



7. Allow 2 minute for setting of cells. After two minutes, starts counting. Only large squares of all the four corners are counted for WBC count. Central ruled small squares are for counting RBCs as shown in figure below. On Neubauers chamber, no letters, numbers and arrows are indicated.
8. Calculate the WBCs.



Letters, numbers and arrows are for illustration only.
Circles depict areas seen through the microscope.

Calculation

1. Count the cells in the Neubauer chamber. These are counted in the four large corner squares labeled as WBC and if the number is Y.

The total area of each the corner large squares is $1mm^2$

Since the depth is $^1/_{10}\ mm$.

Therefore, the volume of each corner large square is $1\ mm^2 \times {}^1/_{10}\, mm = {}^1/_{10}\, mm^3$.

Therefore, the volume of the four corner large squares is

$$^1/_{10} \times 4 = {}^4/_{10}\, mm^3$$

The number of WBCs in $1mm^3$ ……..….. X

The number of WBCs in $^4/_{10}$ ……..…….. Y

Therefore,

$$X = \frac{Number\ of\ WBCs\ in\ all\ large\ squers \times 1}{Volume\ of\ all\ corner\ large\ squares} = \frac{Y \times 1}{4/10} = Y\frac{1}{4/10} = Y \times \frac{10}{4} = Y \times 2.5$$

Since, dilution is 20.

Therefore number of WBC in 1mm^3, i.e. $X = Y \times 2.5 \times 20 = Y \times 50$

Therefore,

$$Total\ number\ of\ WBC = Number\ of\ WBC\ counted \times 50$$

On the other way, the calculation may be adapted as follows:

Since, the total number of WBCs is $\frac{Number\ of\ cells \times Dilution\ factor \times Depth\ factor}{Area\ count}$.

Where dilution factor is 20, depth factor is 10 and area count is 4.

No. of cells X Dilution factor X Depth factor Area count Where: Dilution factor = 20, Depth factor = 10, Area count = 4

2. If the count is low, i.e., below 4000 per mm^3, then use the dilution of 10.

Precautions

- Always wear protective gloves/protective clothing/eye protection/face protection before handling the dilution fluid.
- Follow good microbiological lab practices while handling specimens and culture.
- Standard precautions as per established guidelines should be followed while handling clinical specimens.

Sources of errors

- Presence of microclots in the sample.
- Inadequate mixing of contents.
- Improper filling of the chamber.
- Improper dilution.
- Mistake in the calculation.

Significance

- It helps us to find out whether a person has got any infection, if yes then what type of infection.
- The TLC analysis makes us able to differentiate between acute and chronic infection.
- It helps us to find out certain types of cancer in a patient.
- It helps the physician to follow the patient with chemotherapy.
- It helps in analyzing the effect of drugs.

Bibliography

- Bain B.J., Bates I., Laffan M.A. 2016. Dacie and Lewis Practical Haematolog. Elsevier Health Sciences, Philadelphia, USA
- Kale R.R. & Kale S.R. 2002. Haematology, Practical Human Anatomy and Physiology. Eight Edition. Nirali Prakashan, Pune, India
- Singh T. 2017. Text and Practical Haematology for MBBS. Arya Publications, New Delhi, India