EXPERIMENT NO. 9b

Aim: Enumeration of Total White blood cells (TLC) using haemocytometer.

Learning Objectives: The total number of leukocytes is in thousands per cub millimetre of blood in comparison to erythrocytes which are in millions and hence bloc is diluted only 20 times for counting these cells. Further, leukocytes are made distinct b using stain in the diluting fluid which stains the nucleus so to distinguish them easily fro non-nucleated RBCs.

Requirements:

Materials: Haemocytometer apparatus with a glass slide, cover slip and WBC pipet small Petri dish, dropper, filter paper, light microscope, pricking needle and cotton.

Chemicals: Alcohol, human blood and a diluting fluid for WBCs counting-Turk Fluid.

Composition of Turk's Fluid with Role of each Component

1. 3 ml glacial acetic acid (to lyse the membranes of RBCs and platelets),
2. 1 ml of 1 % Gentian violet (to stain the nuclei of WBCs to deep blue/violet for ea identification and differentiation from dust particle etc.)
3. 100 ml Distilled water (acts as solvent to dissolve all the ingredients).

Principle: Leukocytes or white blood cells (WBCs) are amoeboid, colorless, la nucleated cells. These are fewer in number as compared to RBCs (Normal ratio of WBC RBC is 1:700) and do not contain hemoglobin. The total leukocyte count (TLC) in an ad is 6000-9000/cumm of blood. WBCs are mainly involved in defense mechanisms. Th protect the body from micro-organisms.

Procedure : It is the same as described for enumeration of RBCs except that the bl is sucked up to "0.5" mark and then diluted to mark 11 (20 times dilution) by **Tur fluid** in the WBC pipette containing a white bead in its bulb (**Figure 1**) This result the haemolysis of RBCs leaving behind the intact WBCs with stained nucleus for e dentification and counting. *The same Thoma Zeiss Haemocytometer* as explained Experiment No. 9 is used for enumerating WBCs. The WBCs are counted in outer orner squares marked as A, B, C and D (**Figure 2**).

Figure 1: Pasteur Pipette and charged Haemocytometer for WBC Counting.

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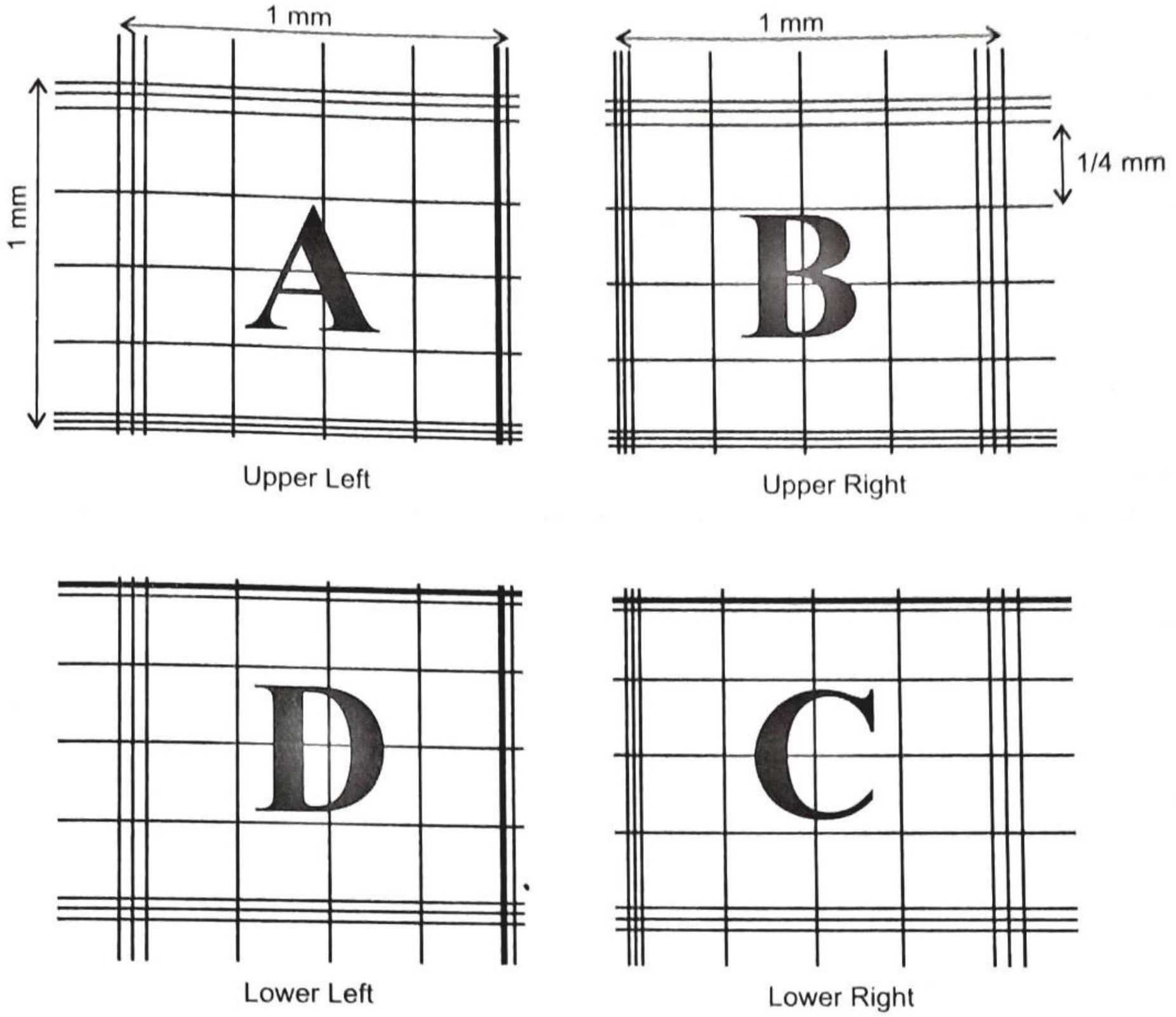

Figure 2: WBC Counting Chambers-A, B, C and D in a Haemocytometer Slide.

Observations: Place the values of WBCs counted in each of the 16 small squares of one big square. Add the counted values of all these 16 squares and put the sum of these below its big square, e.g. of square A below it as W_1. Similarly do for the remaining three squares and put their values below the respective square B, C and D as W_2, W_3 and W_4 respectively. Record the observations in a tabular form as shown in **Table 1.**

Table 1: Recording of observations of counting of WBCs.

Reading	Number of WBCs in four Squares				Total Number of WBCs in Five Squares (W)	Average of two readings (W)
	A	B	C	D		
1st set	W_1	W_2	W_3	W_4	$W = (W_1+W_2+W_3+W_4)$	$(W + W) \div 2 = \mathbf{W}$
2nd set	W_1	W_2	W_3	W_4	$W = (W_1+W_2+W_3+W_4)$	

Calculations:

Area of one WBC square: $= 1 \times 1\ mm^2$

The depth of a square: $= 1/10\ mm$

Volume of one WBC square: $= 1 \times 1 \times 1/10\ mm^3$

Volume of four WBC squares: $= 1 \times 1 \times 1/10 \times 4\ mm^3$

$= 4/10\ mm^3 = 2/5\ mm^3$

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Total number of WBCs counted in 4 squares: $= W = (W_1 + W_2 + W_3 + W_4)$

Average of two sets of readings: = W of 1st set + W of 2nd set ÷ 2 = **W**

The number of WBCs in 2/5 mm^3 = **W**

Therefore, number of WBCs in 1 mm^3 = **W** × 5/2

Since the blood has been diluted 20 times

Thus, number of WBCs in 1mm^3 of blood = **W** × 5/2 × 20

OR = **W** × 50

Results: The number of WBCs in the human blood is expressed in thousands per mm^3 of blood. The normal value of WBCs in human blood varies between 6,000 to 9,000 per mm^3.

Discussions: Since leucocytes perform many functions, maintenance of their normal values in blood becomes essential to remain healthy. Total leucocyte count (TLC) is done with the help of haemocytometer having Neubauer's markings and is expressed in thousands per cubic mm of blood. **Leucocyte** count shows diurnal variations; usually it is low in the morning samples. **Leucocytosis** (increase above normal limits) and **leucopenia** (decrease below normal limits) are two abnormal conditions of leucocyte count. **Leukaemia** is a morbid condition resulting in cancerous growth of WBCs with fatal termination.

Clinical Significance: Normal total leukocyte count is 6000 to 9000 cubic mm of blood in adults. WBC count in children is higher than in adults and at birth, it is 16,000/cubic mm of blood. Abnormal conditions of total WBC count are **leucocytosis** and **leucopenia.** WBC count of more than 11,000/cubic mm of blood is **leucocytosis** and it occurs in pyrogenic infections and inflammatory conditions. WBC count of less than 4000/cubic mm of blood is known as **leucopenia** and it indicates typhoid fever.

Precautions: Same as those undertaken for counting of RBCs.

RELEVANT QUESTIONS

Q.1. What is meant by the term "glacial"? Why should the acid in the Turk's fluid be the glacial?

Ans. The term glacial means pure acetic acid. Only the glacial acid can give the typical shine around the WBCs due to swelling of nuclei and this makes the differentiation of WBCs from dust particles easy.

Q.2. What is the name of WBCs diluting fluid? Give its composition.

Ans. WBCs diluting fluid is called Turk's fluid. The composition of Turk's fluid is- Glacial acetic acid = 1.5 ml, Gentian violet (1% solution in water) = 1.5 ml and Distilled water = 100 ml.

Q.3. How will you differentiate WBC pipette from RBC pipette?

Ans. RBC pipette has a red bead and a larger bulb while WBC pipette has a white bead and a smaller bulb. Further, the markings in RBC pipette are 0.5. 1 and 101 while

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in WBC pipette, these are 0.5, 1 and 11 thus giving a dilution of 200 times and 20 times respectively.

Q.4. Name few of the RBCs lysing chemicals.

Ans. Few of the RBCs lysing chemicals are glacial acetic acid, hydrochloric acid, Triton X-100, saponin, zaponin etc.

Q.5. How does Turk's fluid act?

Ans. Glacial acetic acid in Turk's fluid destroys the RBCs and Gentian violet stains the nucleus of leukocytes so that these can be identified and counted easily.

Q.6. When 0.5 mark blood is diluted to 11 mark in a WBC pipette, why is dilution obtained 20 times and not 22?

Ans. When the blood is diluted from 0.5 mark to 11 mark, the diluting fluid is sucked into the pipette. During this act, blood goes into the bulb and it is followed by the diluting fluid. However, just below the bulb, there is a mark 1 and this diluting fluid remains in the stem of the pipette and does not mix with the blood. Therefore, the fluid up to the mark 1 does not participate in the dilution and the blood up to the 0.5 mark is diluted by a volume of fluid present in the bulb (11-1=10) thus giving 20 times dilution only.

Q.7. Why bulb of WBC pipette is smaller than that of RBC pipette?

Ans. The bulb of WBC pipette is smaller than that of RBC pipette because dilution required for counting of WBCs is much less (only 20 times) than that required for RBCs counting (200 times).

Q.8. What is the volume of one small square in which WBCs are counted?

Ans. Volume of one small square is $1/4 \times 1/4 \times 1/10 = 1/160$ cubic millimeter with each side as 1/4 mm and depth as 1/10 mm.

Q.9. Why is counting started only after waiting for two minutes of charging of the chamber and not immediately?

Ans. Two minutes are required for settling down of WBCs in the chamber and because of this, counting is done only after waiting for two minutes of charging of the chamber and not immediately.

Q.10. How does the WBCs count vary with age?

Ans. WBCs count varies with age. It is about 20,000 per cubic mm of blood at birth. After the second week of birth, the count starts falling and the adult value is reached when the child is between 5-10 years. During the period of infancy, the lymphocytes predominate in the WBCs count. The number varies considerably, even in healthy individuals, the range being 6000-9000 per cubic mm and the average value of WBCs count in an adult is 7000 per cubic mm of blood.

Q.11. Why leukocytes count is important?

Ans. Leukocytes play many important functions because of which their adequate number in the blood becomes essential. Leukocytes have the following functions:

(*i*) Phagocytic action– Mainly performed by neutrophils and monocytes. By this action foreign particles or bacteria are engulfed.

(*ii*) In defense– Lymphocytes manufacture antibodies for defense.

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(*iii*) In tissue repair– In an area of inflammation, lymphoblasts may be converted into fibroblasts, thereby helping in the repair process.

(*iv*) Secrete heparin– Preventing intravascular coagulation.

(*v*) Synthesize globulins– Lymphocytes produce **β** and **α** globulins.

(*vi*) Manufacture trephones– Certain substances which greatly influence the nutrition, growth and repair of tissues are manufactured by leukocytes from plasma proteins. These are called trephones.

(*vii*) Combat allergic reactions– Histamine, eosinophil chemotactic substance, neutrophil chemotactic substance and heparin secreted by leukocytes help in minimizing the severity of allergic reactions.

Q.12. What is leucopenia? State the conditions which lead to leucopenia.

Ans. Leucopenia is a fall in leucocyte count below 4000 cubic mm of blood. The conditions which lead to leucopenia are-

(*i*) Malnutrition, starvation, extreme weakness and debility.

(*ii*) Nonpyogenic infections: Most common in typhoid and paratyphoid and less frequent in influenza, malaria, smallpox, mumps and toxemias.

(*iii*) Drugs: Chloramphenicol used in the treatment of typhoid is known to suppress the bone marrow causing leukopenia.

(*iv*) Repeated exposures to X-rays and radium.

(*v*) Arsenic, antimony and dinitrophenol poisoning depress the bone marrow.

(*vi*) Diseases of blood and bone marrow: Aplastic anaemia, pernicious anaemia and aleukemic stage of leukemia.

Q.13. What is leucocytosis? State the conditions which lead to leucocytosis.

Ans. Leucocytosis is an increase in leucocyte count above 11,000 cmm of blood. The conditions which lead to leucocytosis are-

(*i*) During acute infections (local and general),

(*ii*) During coronary thrombosis,

(*iii*) In malignancy,

(*iv*) After hemorrhage and post-operative cases,

(*v*) During uremia, metabolic acidosis, burns etc.

(*vi*) After poisoning by drugs and chemicals,

(*vii*) After convulsive seizures,

(*viii*) After administration of adrenaline and ACTH,

(*ix*) After muscular exercise,

(*x*) Due to stress and emotional strains.

(*xi*) During pregnancy.

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