

ESTIMATION OF WHITE BLOOD CELL COUNT (TLC/ Total Leucocyte Count)

Background:

White blood cell count is an enumeration of white corpuscles or is an leucocyte count.

The white blood cells (WBCs) or leucocytes are nucleated actively amoeboid and do not contain haemoglobin and are originated purely from extravascular tissue. They are composed of nucleoproteins and varieties of enzymes. Their number is less and life span is short as compared to red blood cells. The WBCs exist in two forms viz. granulocytes and agranulocytes. Granulocytes are further classified as eosinophil, basophil, neutrophil, while agranulocytes shows lymphocytes and monocytes. These varieties possess independent morphological, functional and staining properties. The main function of white blood corpuscles is phagocytosis that is body defence mechanism against foreign particles and invading bacteria. They are also involved in antibody formation in immunological body defence mechanism. It also take part in process of repair in an area of inflammation.

The basic principle is that the blood is diluted with acid solution which removes the red cells by haemolysis and also accentuates the nuclei of the white cells; thus the counting of the white cells becomes easy.

Blood cell counts can be performed using the **hemacytometer**.

Significance: The white cell count is the number of white cell present in one cubic millimeter of blood. The normal values of white blood cell count varies between 5000 to 10,000 per cubic millimeter or 7-11 thousand cells/ μ l of blood volume in healthy individual. Variation in normal values is observed in diseased states. WBC count increases (leucocytosis) in conditions like pneumonia, leukemia, meningitis, small pox etc. while the count decreases (leucopenia) in conditions such as influenza, typhoid, infectious hepatitis etc. Moreover the count rises in pregnancy and during menstruation. Thus, white blood cell count is useful in diagnosis.

The aim of the experiment is to estimate white blood cell count of a blood specimen.

REQUIREMENTS:

Neubaur chamber, WBC pipette, Cover slip, WBC diluting fluid, Needle, spirit, cotton.

PROCEDURE:

1. Sterilize the finger tip with cotton plug soaked in 70% alcohol and let it dry.
2. Take a bold prick to have free flow of blood and draw the blood in a WBC pipette up to 0.5 mark.
3. Dip the WBC pipette in WBC diluting fluid up to 11 mark and rotate the pipette equally in your hands to mix the solution well by swirling.
4. Take the haemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.
5. Allow a small drop of diluted blood, hanging from the pipette, to sweep into the counting chamber by capillary action. Make sure that there is no air bubble and there is no overfilling beyond the ruled area.
6. Leave the counting chamber on the bench for 3 minutes to allow the cells to settle. Observe the cells by placing the counting chamber on the mechanical stage of the microscope.

Focus on one of the corner squares of the counting chamber and count the white cells schematically, starting from the upper left small square of each Square. Repeat the count in all the four corners of the chamber. Apply the margin rules i.e. count the cells lying on two adjacent margins, and discard those on the other two margins.

DATA ANALYSIS:

$$\frac{\text{No. of cells} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area count}}$$

Where:

Dilution factor = 20, Depth factor = 10, Area count = 4

RESULT: The number of white blood cells present in one μl of bloodspecimen is

REFERENCE:

1. Haematology, Practical Human Anatomy And Physiology, S.R. Kale et al., Nirali Prakashan, Eight Edition, 2002, pp. 16-22