**Total WBCs and differential Leukocytes count**

Total Leukocyte and differential leukocyte count determines the presence of an infection or leukemia. Percentage of each of differential WBC counts. It also monitors the body's reaction to various treatments, checks bone marrow function and general health examinations and to help investigate a variety of illnesses.

White blood cells (WBCs) are also called *leukocytes*, they are commonly amoeboid (cells that move or feed by means of temporary projections, called pseudopods (false feet)). They are formed partially in the bone marrow (*granulocytes* and *monocytes* and a few *lymphocytes*) and partially in the lymph tissue (*lymphocytes* and *plasma cells*). After formation, they are transported in the blood to different parts of the body where they are needed. There are five kinds of WBCs; all are larger than RBCs and have nuclei when mature. The nucleus may be in one piece or appear as several lobes or segments.

In response to an acute infection, trauma, or inflammation, white blood cells release a substance called *colony-stimulating factor* (CSF). CSF stimulates the bone marrow to increase white blood cell production (*leukopoiesis*). Leukocytes can leave the vascular system and enter the connective tissues of the body by squeezing through capillaries (a process known as *diapedesis* or *extravasation*). During an inflammation response, the release of histamine from tissue mast cells and basophiles increases the permeability of the capillaries, and consequently promotes the process of diapedesis. This sequence of events also produces the local edema, redness, and pain associated with inflammation. These special cell help our bodies defend itself against invasion of pathogens. Not only do they help our immune system but they remove toxins, wastes and abnormal or damaged cells.

There are normally 4000 to 10,000 leukocytes (white blood cells) per microliter of human blood.

**Clinical significance:** Increase in total leukocyte count of more than 10000/mm3 is known as leukocytosis and decrease of less than 4000/mm3 as leukopenia.

**Causes of leukocytosis:**

* Anemia and leukemia
* Inflammation (rheumatoid arthritis or allergy)
* Severe emotional or physical stress
* Tissue damage (burns)

**Causes of leukopenia:**

* Bone marrow failure (tumor)
* Disease of the liver or spleen
* Radiation (X-ray) and chemotherapy
* Infections

A. Bacterial (typhoid, paratyphoid, tuberculosis)

B. Viral (hepatitis, influenza)

C. Protozoal (malaria)

**Requirements**

1. Microscope

2. Improved Neubauer Chamber (Hemocytometer) with cover-slip.

3. WBC pipette (The pipette has 3 markings 0.5, 1, and 11).

4. WBC diluting fluid (Turk's solution): It is composed of Glacial acetic acid (1.5ml) + 1% (w/v) Gention violet (1.0ml) + 97.5ml Distilled Water. Filter the solution.

***Procedure***

1. Draw blood up to **0.5** mark of a WBC pipette.

2. Draw diluting fluid up to **11** mark.

3. Mix the contents in the pipette by circular motion and after **2** minutes by discarding few drops, fill the counting chamber and allow the cells to settle for **2** to **3** minutes.

4. Focus on one of the corner squares "WBC marked area" (each having 16 small squares) by turning objective to low power (10X).

5. Count cells in all four WBC marked corner.

6. Calculation:

**Number of white cells/mm3 of whole blood** = No. of white cells counted (N) **X** Dilution (20) **/** (area counted "4" **X** depth of fluid "0.1")

**Number of white cells/mm3 of whole blood** = **N X 20/ (4 X 0.1) = N X 50**

General inflammatory and immune functions of these cells include:

* Destruction of invading microorganisms (bacteria and viruses).
* Identification and destruction of cancer cells.
* Phagocytosis of tissue debris including dead and injured cells

Five types of leukocytes are classified as either granulocytes or agranulocytes:

1. Granulocytes (Contain cytoplasmic granules, generally spherical in shape):
2. Neutrophils
3. Eosinophils
4. Basophils
5. Agranulocytes (Lack granules, Spherical or kidney shaped nuclei):
6. Monocytes

2. Lymphocytes

**Slide Staining with Wright's Stain**

|  |  |
| --- | --- |
|  | Lymphocytes |
| ***Neutrophil*** | ***Lymphocyte*** |
|  |  |
| ***Eosinophil*** | ***Monocyte*** |
|  |  |
| ***Basophil*** |  |

**Slide Staining with Wright's Stain**

1. Place a small drop of whole blood on a very clean slide (near one end).

2. Spread the drop by using another slide (spreader), placing the spreader at a 45° angle and backing into the drop of blood. The spreader catches the drop and it spreads by capillary action along its edge. Now, push the spreader across the slide; this pulls the blood across to make the smear. Smears should be air-dried.

3. using a dropper, cover the slide with enough Wright's stain to completely cover the slide with a layer of stain. Count the number of drops used.

4. After 2 minutes, cover the slide with an equal amount of distilled water to dilute the stain. Allow to stand for 4 minutes.

5. After 4 minutes, wash the slide gently for 30 seconds with tap water.

6. Allow slide to dry at room temperature before examination.

7. Examine the smear, first under low power then under oil immersion, count and identify 100 WBCs.