

## Differential WBC count

**Introduction & principle:** The differential WBC count is performed to determine the relative number of each type of WBC in the blood. In disease states a particular white blood cell type shows increase in number in the blood.

### Methods

1. Manual method
2. Electronic cell counter

### Manual differential white cell count material and instruments

1. Whole blood using EDTA as anticoagulant or capillary blood drawn from a finger or toe puncture.
2. Glass slide
3. Microscope
4. Alcohol 70%
5. Lancet
6. Leishman's stain – its composition:

Leishman's stain	0.15 g
Methyl alcohol	100 ml

7. Immersion oil.

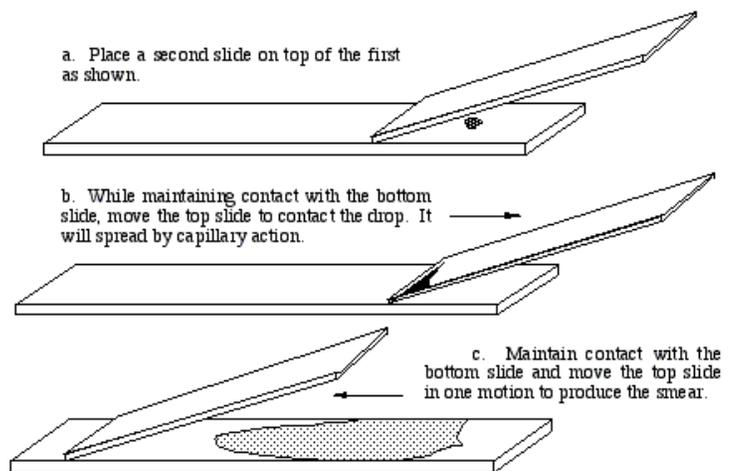
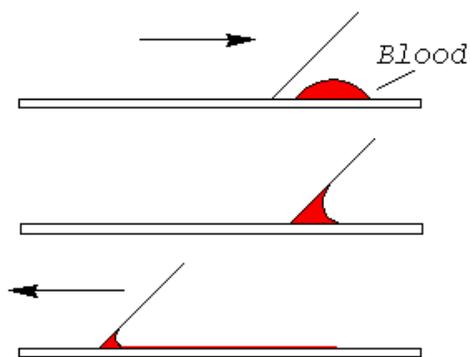
### Composition of Leishman's Stain

Eosin → An acidic dye, which stains the basic protoplasmic material.

Methylene blue → A basic dye, which stains the acidic nuclear chromatin.

Acetone-free methyl alcohol → To fix the smear to the slide. Acetone being a strong lipid solvent it tends to damage the cell membrane. The stain is prepared by dissolving 750 mg of Leishman's powder in 500 mL of acetone-free methyl alcohol.

### Procedure



❖ Prepare the blood smear

- Clean two slide, one to be covered with the blood film and one to be used as spreader.
- Clean the finger with alcohol, allow it to dry and then prick it with a disposable lancet to obtain a drop of blood.
- Make a fine touch of one end of a slide with the drop of blood (only a small amount is required).
- Place the edge of the other slide on the surface of the first slide just in front of the drop of blood and at an angle of 45°.
- Draw the spreader back until it makes contact with the drop of blood.
- Push the spreader slowly and smoothly to the other end of the slide in one motion.
- Allow the film to dry at room temperature i.e. the blood smears should be air-dried
- They should be also labeled immediately with the student's name and the date at the end of slide.

❖ **Staining the blood smear by:**

- Put the dried slide on a staining rack.
- The blood smear should be stained as soon as possible certainly within 1 to 2 hours.
- Carefully drop Leishman's stain onto the blood film until the film is covered.
- Allow the stain to act for one to two minutes.
- Add distilled water to the stain, this gives dilution of 1:1 or 1:2
- The diluted stain should act for 15-30 minutes
- Then wash it off with distilled water, continue washing until the film has a pink color.
- Shake off excess water and allow it to dry at room temperature.

❖ **Examination of the stained smear**

- For examining the blood smear a microscope with a low-power objective (10 x) and an oil immersion objective (100 x) is necessary.
- Place the slide (smear side up) on the microscope stage.
- Examine the blood smear using the low power (10 x) objective. Choose an area where there are plenty of WBCs. This area is usually located near the wedge shaped end of blood smear.
- Place a drop of immersion oil on the selected site and carefully change to the oil immersion objective (100 x)
- Perform the differential cell count and, at the same time examine the morphology of the WBCs.

A good smear should:

1. Be buff colored.

2. Be uniform.
3. Be broader at the head and taper off into a tail.
4. Occupy the middle-third of the slide leaving a margin of about 5 mm along the edges.
5. Have no longitudinal or transverse striations or windows.
6. Have no stained granules or precipitates.
7. Have discrete red cells without overlapping each other.

## Calculation

Count each WBC seen and record on a differential cell counter until 100 WBCs have been counted. For instance, if 25 of the 100 WBCs were lymphocytes, then the percentage of lymphocytes is 25% & so on.

**The normal range percentage of the different types of WBCs is as follows:**

<b>Neutrophils</b>	<b>50-70%</b>
<b>Eosinophils</b>	<b>1-4%</b>
<b>Basophils</b>	<b>0.4%</b>
<b>Monocytes</b>	<b>2-8%</b>
<b>Lymphocytes</b>	<b>20-40%</b>

## Neutrophils

**Neutrophilia** is an increase in neutrophils. **Neutropenia** is a decrease in neutrophils.

### Common Causes of Neutrophilic Leukocytosis:

1. Acute pyogenic infection such as tonsillitis, appendicitis, pneumonia
2. Tissue necrosis as in myocardial infarction
3. Following hemorrhage
4. Trauma, postoperative burns
5. Hemolysis
6. Malignant neoplasm, Myeloproliferative disorders (bone marrow hyperactivity diseases).
7. Metabolic disorders like gout, diabetic acidosis, uremia.
8. Drugs such as glucocorticoids, adrenaline, digitalis, phenacetin
9. Poisoning with lead, mercury, insect venom.
10. Physiological: Exercise, stress, after meals, pregnancy and parturition, nervousness.
11. Inflammatory disorders (non-infectious) such as rheumatic fever, rheumatoid arthritis.

### Neutropenia: Is decrease in the number of neutrophils.

1. Typhoid and paratyphoid fever, kala-azar
2. Viral infection as measles, influenza.
3. Depression of bone marrow due to irradiation, bone marrow diseases and chemotherapy.
4. Drugs such as chloramphenicol

5. Autoimmune diseases. Increased destruction of neutrophils can be due to the body's immune system targeting neutrophils for destruction. This may be related to having autoimmune diseases, such as systemic lupus erythmatosus It can be also due to large spleen (hypersplenism).
6. Nutritional such as anorexia nervosa and in starvation.

## **Eosinophils**

### **Causes of Eosinophilic Leukocytosis (increase eosinophil):**

1. Allergic conditions like asthma, hay fever, urticarial, eczema
2. Parasitic infestations—such as trichinosis, schistosomiasis, hookworm infestation
3. Tropical eosinophilia
4. Collagen disorders
5. Chronic myeloid leukemia
6. Hodgkin's disease.
7. Other disease states: Such as scarlet fever, acute rheumatic fever.

## **Basophils**

### **Causes of basophilia include:**

1. Myeloproliferative disease and hemolytic anemia.
2. Allergic reactions
3. Chicken pox, small pox.

## **Monocytes**

### **Causes of Monocytosis (increased monocytes)**

1. Chronic infection like tuberculosis, syphilis, subacute bacterial endocarditis, brucellosis, malaria.
2. Protozoal infections like malaria, kala-azar
3. Infectious mononucleosis
4. Monocytic leukemia and multiple myeloma
5. Collagen diseases
6. Granulomatous diseases like sarcoidosis, ulcerative colitis.

### **Monocytopenia: Decrease in the number of monocytes occurs rarely in:**

1. Bone marrow failure
2. Aplastic anemia
3. Septicemia.

## **Lymphocyte**

**Causes of Lymphocytosis** (increased number/percentage of lymphocytes): It is of two types:

**[A] Relative:** Total number of circulating lymphocytes unchanged but WBC count due to neutropenia. Relative lymphocytosis is normal in infants and children (aged 4 months - 4 years). Causes of relative lymphocytosis include:

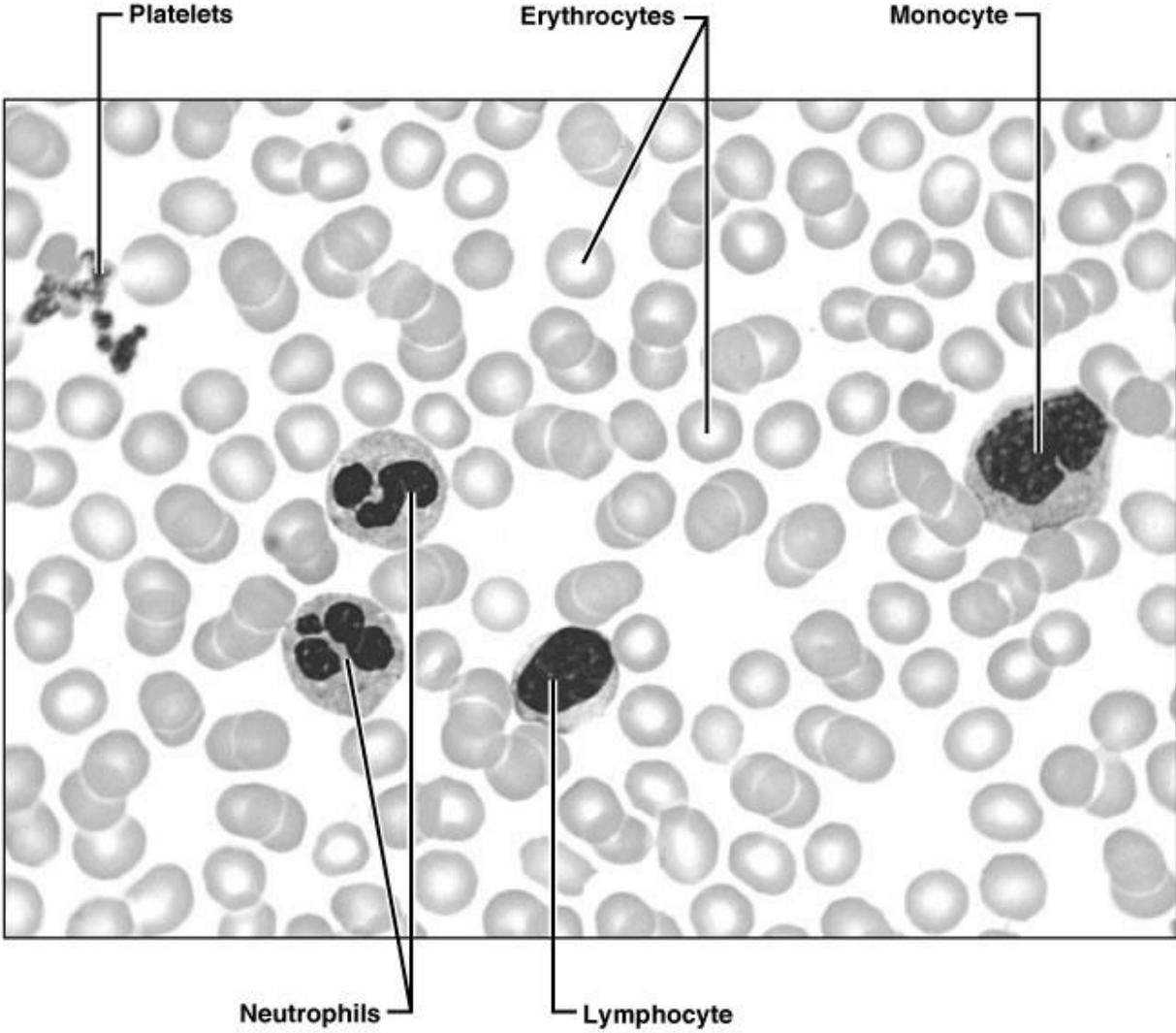
- Age less than 2 years;
- Acute viral infection, and
- Splenomegaly with splenic sequestration of granulocytes.

**[B] Absolute:** Number of lymphocytes increases.

1. Specific acute bacterial infection such as Whooping cough
2. Specific chronic infections like TB, syphilis and malaria
3. Specific protozoal infections, such as toxoplasmosis
4. Viral infection like chicken pox
5. Lymphocytic leukemia

**Causes of lymphopenia**\_(decreased number of lymphocytes):

1. Steroid therapy
2. Radiation (lymphocytes are most sensitive to whole body irradiation)
3. **Characteristic of AIDS**
4. Recent temporary infection such as common cold.

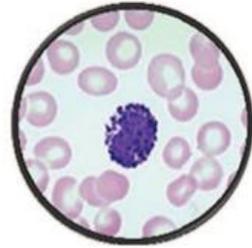




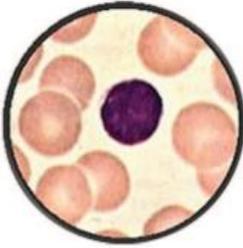
**Neutrophil**



**Eosinophil**



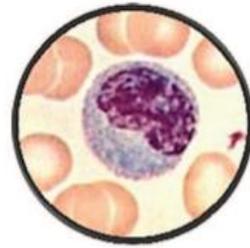
**Basophil**



**Small lymphocyte**



**Large lymphocyte**



**Monocyte**