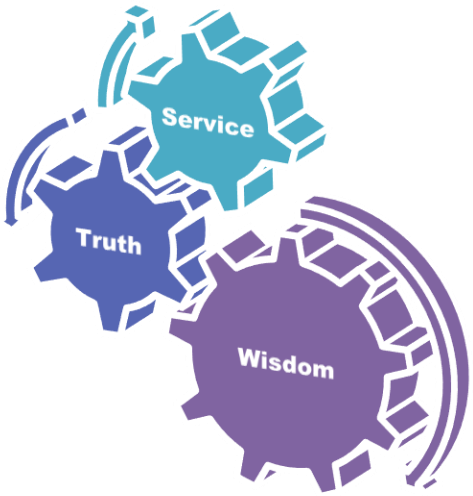
MSK- 1 MODULE
SKILL LAB /Physiology PRACTICAL
FIRST YEAR MBBS BATCH 50

Dr .Afsheen Batool

Date: 6th April2023

Determination of Differential Leukocyte Count(DLC)

Motto



Vision; The Dream/Tomorrow

- To impart evidence based research oriented medical education
- To provide best possible patient care
- To inculcate the values of mutual respect and ethical practice of medicine

LEARNING OBJECTIVES

At the end of skill lab , students must be able to:

- Understand what is differential leukocyte count?
- Determine types of white blood cells
- Understand leukopoiesis
- Describe characteristic features of white blood cells.
- Understand functions of WBCs.
- Perform method for determination of white blood cells.
- Know precautionary measures to perform DLC.

INTRODUCTION

- **DLC** is defined as the number of different type of leukocytes present in one hundred leukocytes counted. It is represented as percentage %.

Types of white blood cells:

1. **Granulocytes:**

a)Polymorphonuclear Neutrophils

b)Polymorphonuclear Basophils

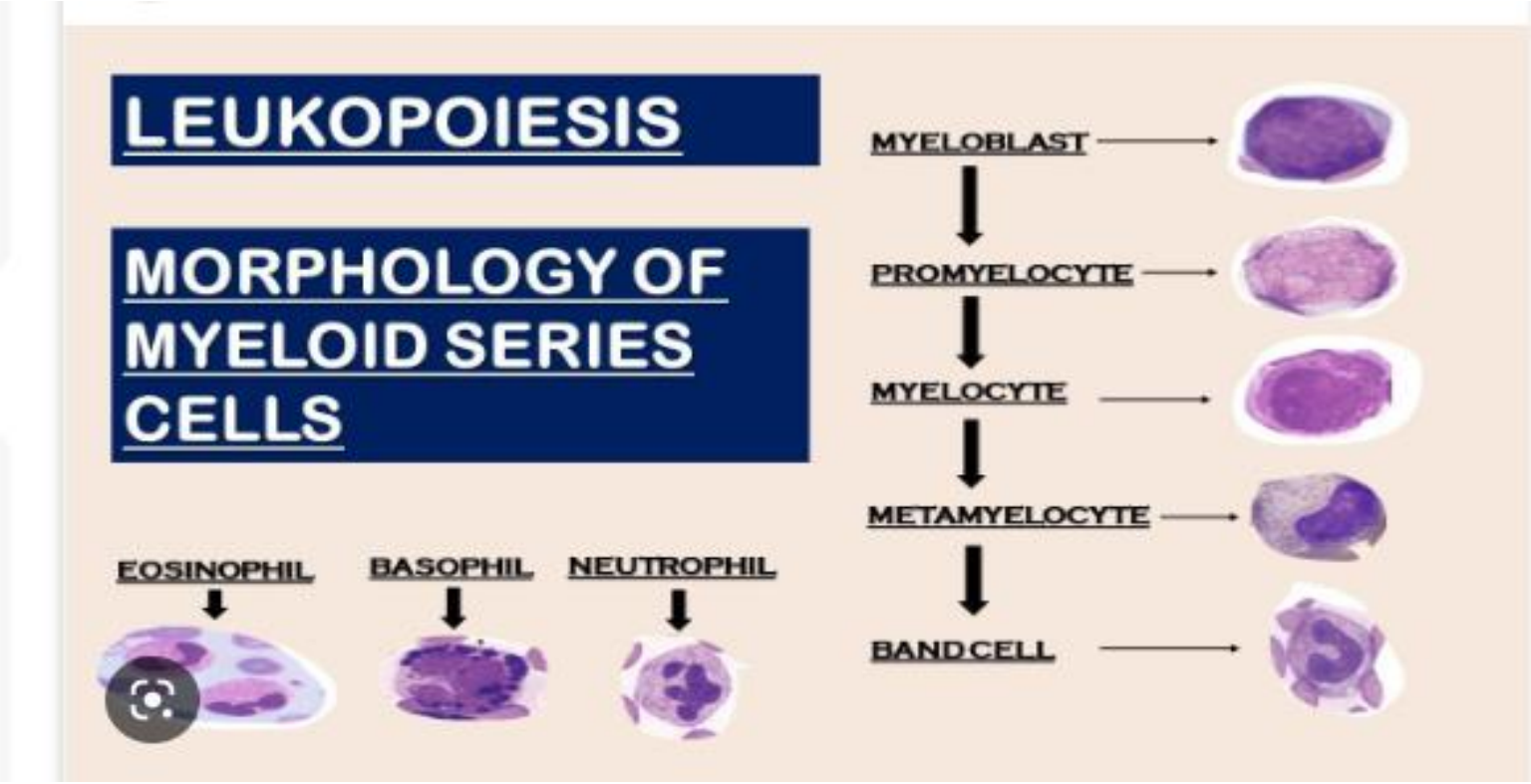
c)Polymorphonuclear Eosinophils

2. **Agranulocytes:**

a)Monocytes

b)Lymphocytes

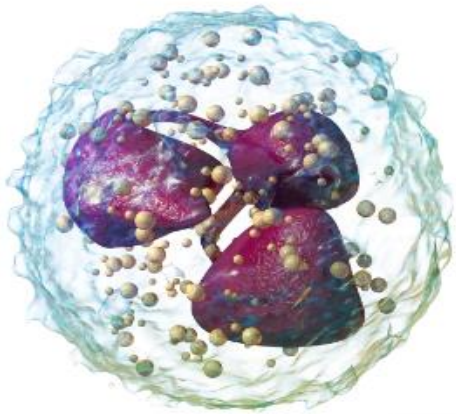
LEUKOPOIESIS

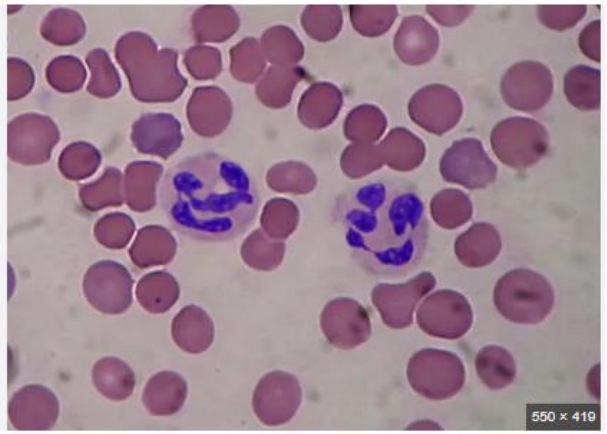


Reference:https://www.google.com/search?q=+leukopoiesis&tbm=isch&ved=2ahUKEwji

Characteristic features of white blood cells

- **NEUTROPHILS:**
- 60 to 70% of all WBCs.
- 10 to 12um diameter
- Nucleus has 25 lobes connected by thin strands of chromatin.
- Cytoplasm has very fine ,pale lilac granules.

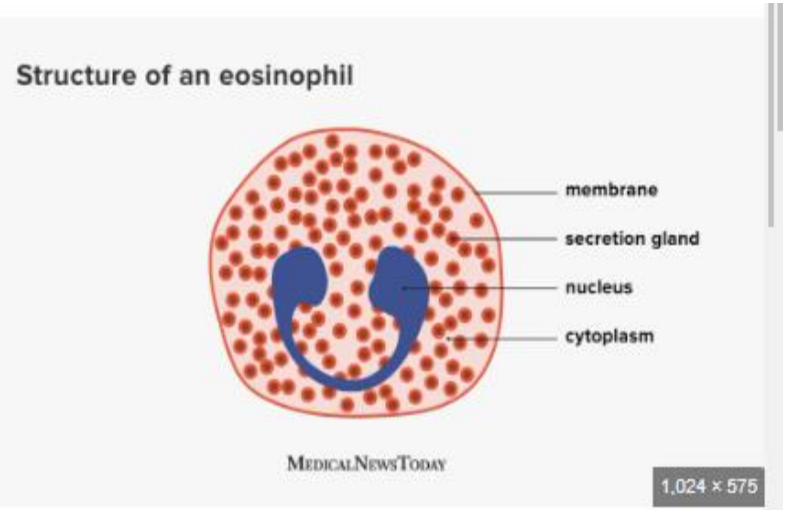


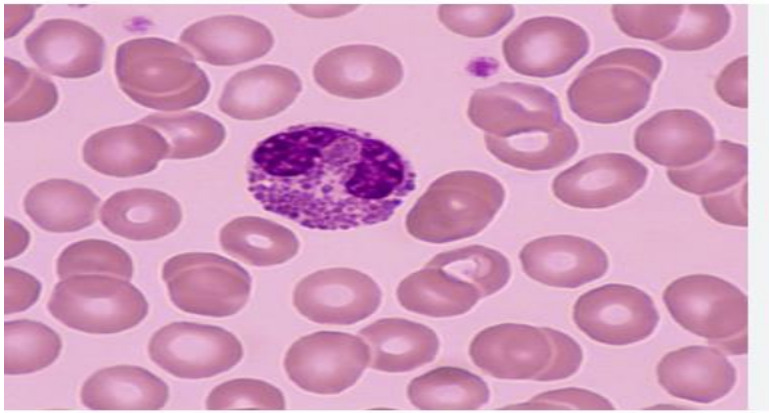


Reference:https://www.google.com/search?q=netrophils&oq=netrophils&aqs=chrome

CONTD….

- **Eosinophils**:
- 2 to 6% of all WBCs.
- 10 to 12um diameter.
- Nucleus has 2 lobes connected by a thick strand of chromatin.
- Cytoplasm is filled with large red-orange granules





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Reference:/www.google.com/search?q=eosinophils&oq=eosino&aqs=chrome.1.69i57j0i433i512j0ihttps

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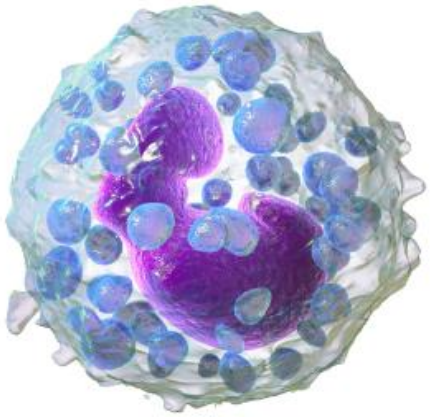
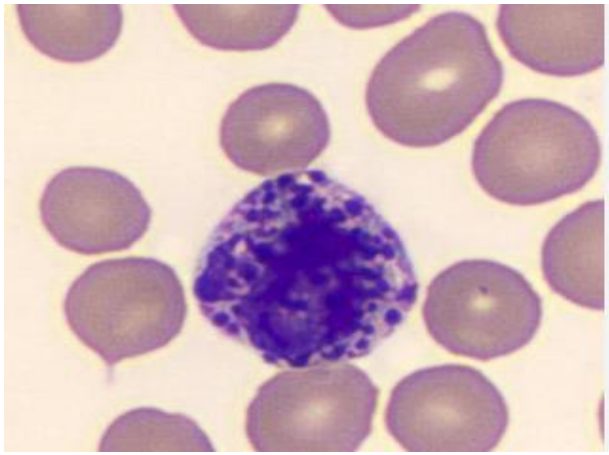
- **Basophils:**
- 0.5 to 1% of all WBCs.
- 8 to 10um diameter.
- Nucleus has two lobes.
- Large cytoplasmic granules appear deep blue purple.





Reference:https://www.google.com/search?q=basophils&source=lnms&tbm

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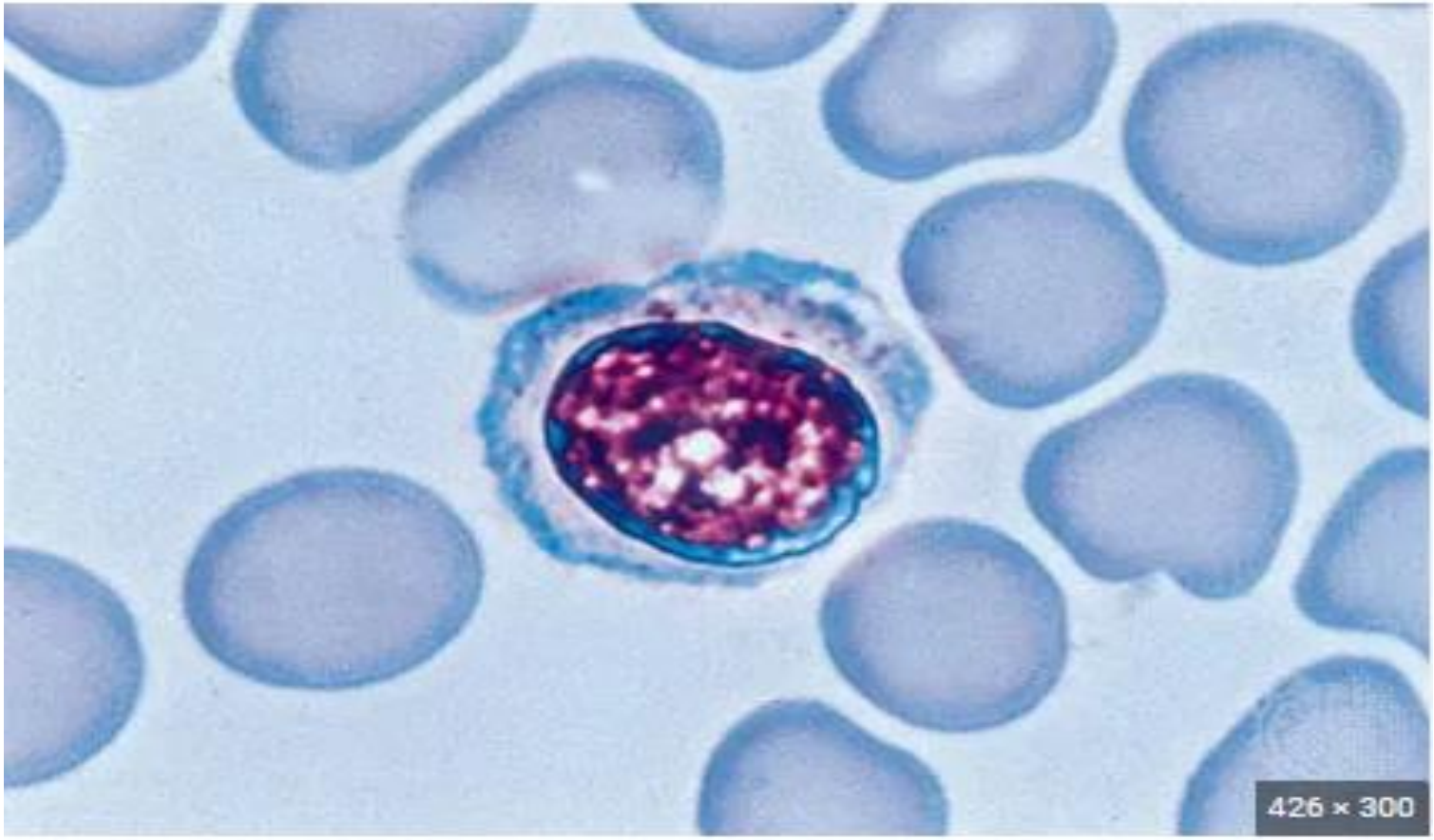
- **Lymphocytes(T** cells and **B** cells):
- 20 to 25% of all WBCs.
- Small lymphocytes are 6 to 9um in diameter.
- Large lymphocytes are 10 to 14um in diameter.
- Nucleus is round or slightly indented.
- Cytoplasm forms a rim around nucleus that looks sky blue ;the larger the cell ,the more cytoplasm is visible.

Lymphocytes



Reference:https://www.google.com/search?q=lymphocytes&hl=en&source=lnms&tbm

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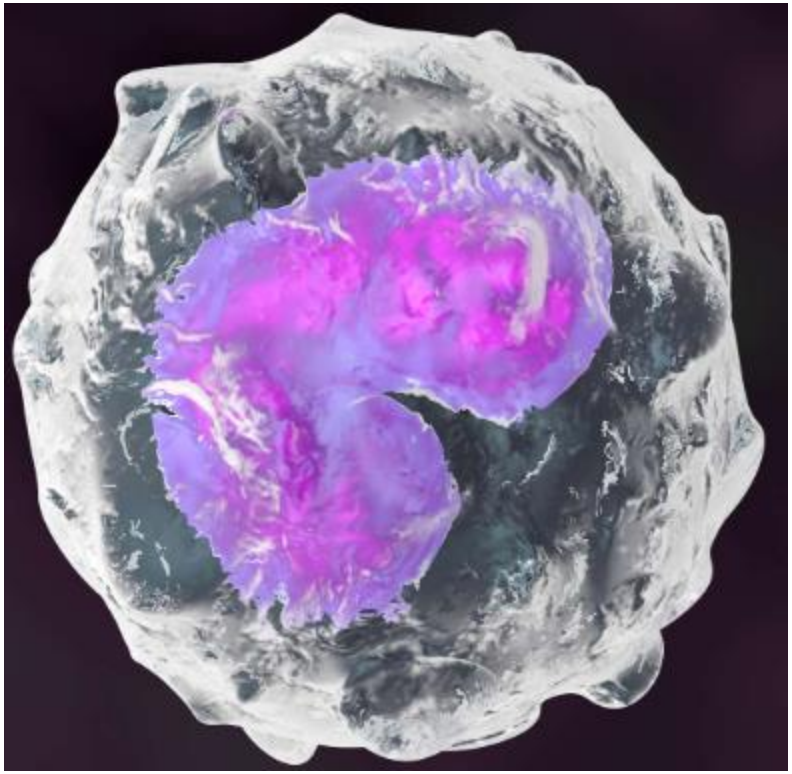
- **Monocytes:**
- 3 to 8 % of all WBCs.
- 12 to 20um diameter.
- Nucleus is kidney shaped or horseshoe shaped.
- Cytoplasm is blue-grey and has foamy appearance.



Reference:https://www.google.com/search?q=monocytes&source=lnms&tbm

FUNCTIONS OF WHITE BLOOD CELLS

- **Neutrophils:**

  Main function is phagocytosis.
- Destruction of bacteria with lysozyme ,defensins and strong oxidants such as superoxide anion ,hydrogen peroxide and hypochlorite anion.
- **Eosinophils:**

  Combat the effects of histamine in allergic reactions. Phagocytize antigen -antibody complexes and destroy certain parasitic worms.

CONTD…

- **Basophils:**

  Liberate heparin ,histamine and serotonin in allergic reactions that intensify the overall allergic response.

- **Lymphocytes:**

  Mediate immune responses including antigen antibody reactions .B cells develop into plasma cells which secrete antibodies. T cells attack invading viruses ,cancer cells and transplanted tissue cells.

- **Monocytes:**

  Main function is phagocytosis.

METHOD FOR DETERMINATION OF WHITE BLOOD CELLS

Principle:

A blood film is stained with leishman's stain and scanned under oil immersion ,from one end to the other .

- As each WBC is encountered ,it is identified until 200 leukocytes have been examined.
- The percentage distribution of each type of WBC is then calculated.
- Knowing the TLC and the differential count ,it is easy to determine the number of each type of cell perm

CONTD…

- **Apparatus and Material:**

  Microscope. Glass slides. Sterile lancet. Cotton. Spirit. 70% alcohol. Glass dropper. Leishman's stain.

- **Composition of Leishman's stain:**

  Constituents of Leishman's stain are:

1. **Eosin:** it is an acidic dye(negatively charged) and stains basic(positive) particles granules of Eosinophil.
2. **Methylene blue:** it is a basic (positively charged)dye and stain acidic(negatively charged)granules in the

CONTD…

cytoplasm, nuclei of leukocytes .It gives blue violet colour to the granules of basophils.

3. Acetone- free and water- free absolute methyl alcohol : It serves two functions:

a) It fixes the blood smear to glass slide.

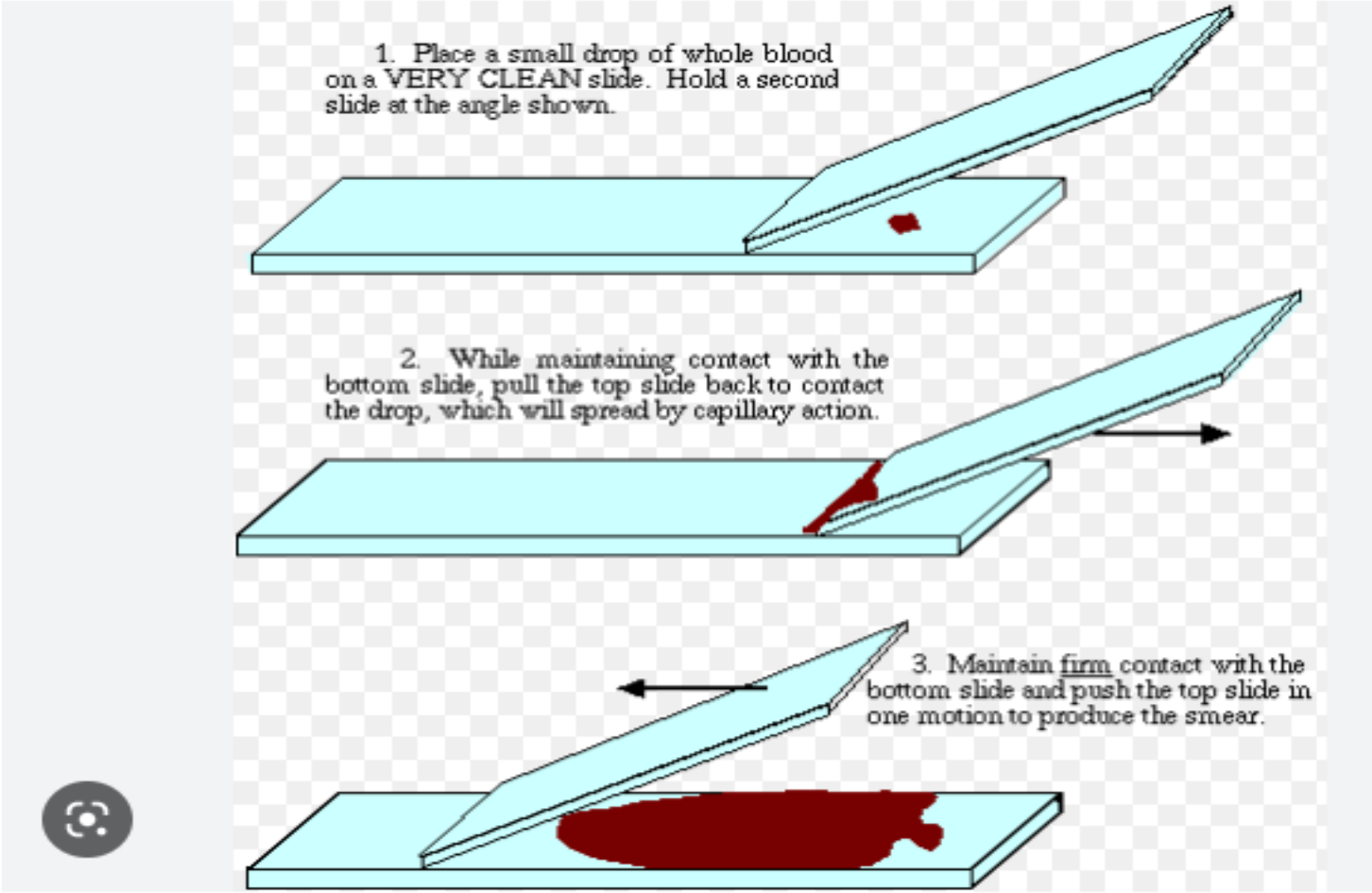
. The alcohol precipitates the plasma proteins, which then act as glue which attaches (fixes) the blood cells to the slide ,so that they are not washed away during staining.

b) The alcohol preserves the morphology and chemical status of the cells.

PROCEDURE

A. Preparing the blood films:

1. Clean the fingertip with spirit swab and prick the finger with lancet.
2. Wipe off first drop of blood and place drop of blood near the edge of glass slide.
3. Spread the drop quickly over the glass slide with the help of spreader slide. keep the spreader slide at an angle of 45 degrees while spreading the blood. Prepare 3-4 such slides.
4. Air dry the slides immediately by waving them in the air.



Reference:https://www.google.com/search?q=preparation+of+blood+film&source=lnms&tbm=isch&

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b. Fixing and staining the blood film:

1. Place the slides on a staining rock or on glass rod stand.
2. Pour 8-10 drops of stain on each unfixed slide by dripping it from a drop bottle, or use a dropper to cover blood films and note the time.
3. Allow the stain to remain undisturbed for 1 to 2 minutes.
4. After the fixing time is over, add an equal number of drops of distilled water through a dropper without scratching

CONTD…

the smear. A glossy greenish layer(scum)soon appears on the surface of the diluted stain

5. Allow the diluted stain to remain on the slide for 6 to 8 minutes.
6. Flush off the diluted stain in a gentle stream of distilled water for about 30 seconds and leave the slides on the rack for about a minute with the last wash of water covering them.
7. Drain the slides and put them in an inclined position against a support.

FEATURES OF A WELL STAINED BLOOD SMEAR

a) **Naked eye appearance:**

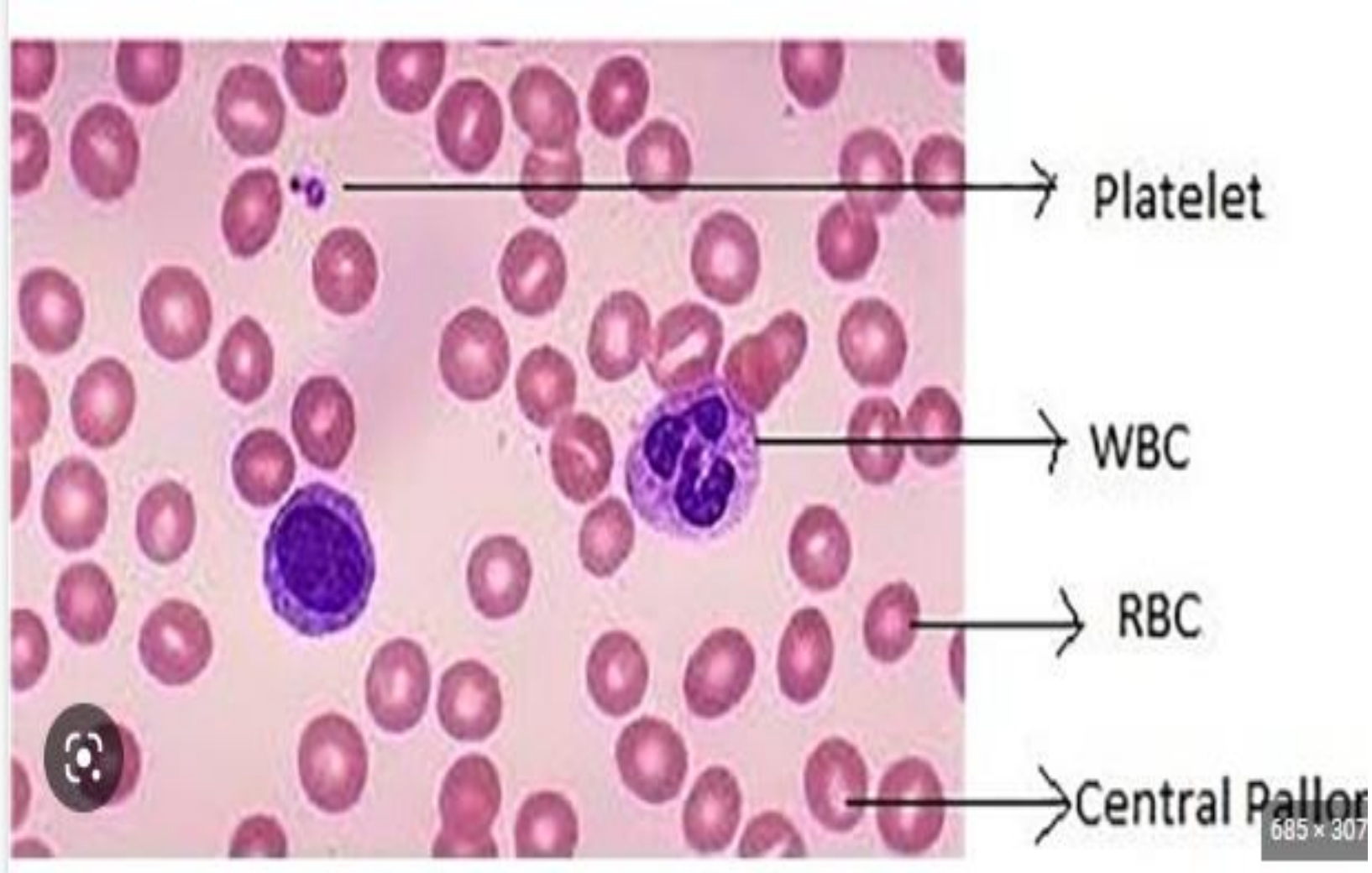
The smear appears translucent and bluish-pink against a white surface ,its thickness being uniform throughout.

b) **Under microscope:**

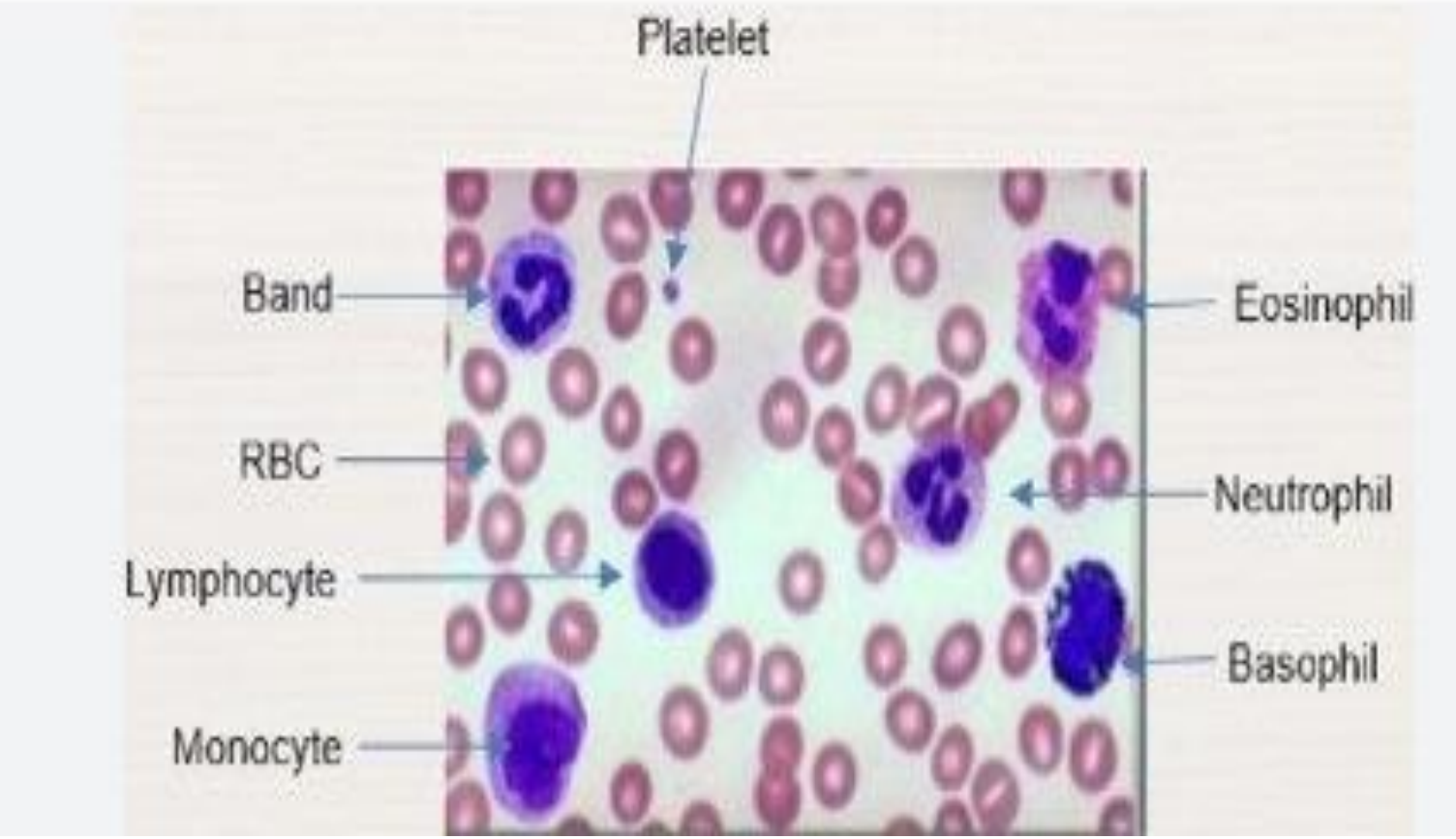
The red cells are stained dull orange-pink and show a central pallor(due to biconcavity)which, if wide may give appearance of rings.

The WBCs show deep blue-violet nuclei and lie unevenly here and there among the red cells.

The platelets occur in small groups



Reference:https://www.google.com/search?q=blood+smear&oq=blood+smear&aqs=chrome.



Reference:
https://www.google.com/search?q=blood+smear&oq=blood+smear&aqs=chrome.

DIFFERENTIAL COUNTING OF LEUKOCYTES

1. Blood film has three parts; tail, body and head. Select a well stained area in the blood film.
2. Draw 200 squares or 100 squares in your workbook for recording various WBCs as they are encountered and identified one after another. Enter these by using the letters N for Neutrophils,M for Monocytes, E for Eosinophils ,B for Basophils and L for Lymphocytes .
3. Place 2 to 3 drops of Cedar Wood oil over the selected portion and focus the slide under oil immersion lens .Bring oil immmersion lens into position till it enters the oil drop.adjust the focus.

CONTD….

4. Move the slide slowly from right to left and as you encounter a leukocyte ,identify and enter it into your workbook.

5. After finishing counting ,count the leukocytes once more starting from lower left corner of the film.

6. Calculate percentage of each type of cell when counting is complete.

PRECAUTIONS

1. The blood film should be thin and smooth.
2. The slides must be clean.
3. Staining should be done carefully .Avoid over and under staining.
4. The lens and slide contact must be carefully guarded to avoid the breaking of oil.

QUESTIONS

1. What is neutrophilia and mention its physiological causes?

Answer:

Increase in number of neutrophils is called neutrophilia.

Causes: physiological:

a)Pregnancy b)Emotional stress

c)Menstruation and lactation

d)Newborn babies.

QUESTIONS

2. What are the causes of eosinopenia?

Answer:

a) Steroids

b) Stressful conditions

c) Acute pyogenic infections

MCQS

- **A 4 years old child presented with complaints of abdominal pain and decrease appetite .His mother also gave history of eating soil. Diagnosis of worm infestation was made .which of the following cells would be increased?**

a) Neutrophils
b) Eosinophils
c) Basophils
d) Lymphocytes
e) Monocytes

Answer

b)EOSINOPHILS

LEARNING RESOURCES

- Practical physiology 1st year MBBS by DR Saqib Sohail.
- Guyton and hall textbook of medical physiology 14th edition.
- Google images.

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