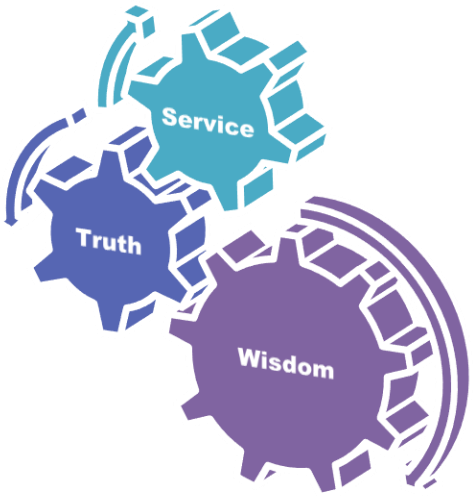
MSK- 1 MODULE
SKILL LAB /Physiology PRACTICAL
FIRST YEAR MBBS BATCH 50

Date: 6th May 2023

DETERMINATION OF RED BLOOD CELL COUNT

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:

1. Describe the relevance of doing red cell count.
2. Identify the RBC pipette; fill it with blood and diluent.
3. Charge the counting chamber and count the red cells.

LEARNING OBJECTIVES

4. Describe the composition of diluting fluid and the function of each component.
5. Give the normal RBC count in different age groups. 6. Describe the site and states of erythropoiesis, and factors that regulate it.

6. 7. Explain the causes of anemia and polycythemia

INTRODUCTION

- Red blood cells (RBCs), also known as erythrocytes, are the most abundant type of cell in the human body, accounting for about 25% of all cells.
- They are an essential component of the circulatory system and are responsible for transporting oxygen from the lungs to the body's tissues and carrying carbon dioxide from the tissues back to the lungs to be exhaled.

INTRODUCTION (Cont.)

- RBCs are unique in their shape, being disk-like with a concave center, which increases their surface area and allows for more efficient gas exchange.
- They are produced in the bone marrow and have a lifespan of approximately 120 days before being broken down and recycled by the body. RBCs play a critical role in maintaining overall health and well-being, and abnormalities in their function or production can lead to a range of serious medical conditions.

CHARACTERISTIC FEATURES OF RED BLOOD CELLS (Cont.)

- Here are some characteristic features of red blood cells:
- **Shape:** Red blood cells are biconcave disc-shaped, which gives them a large surface area-to-volume ratio, facilitating gas exchange.
- **Lack of nucleus:** Unlike most other cells in the body, red blood cells lack a nucleus and other organelles. This allows more space for hemoglobin, the protein that carries oxygen and carbon dioxide in RBCs.

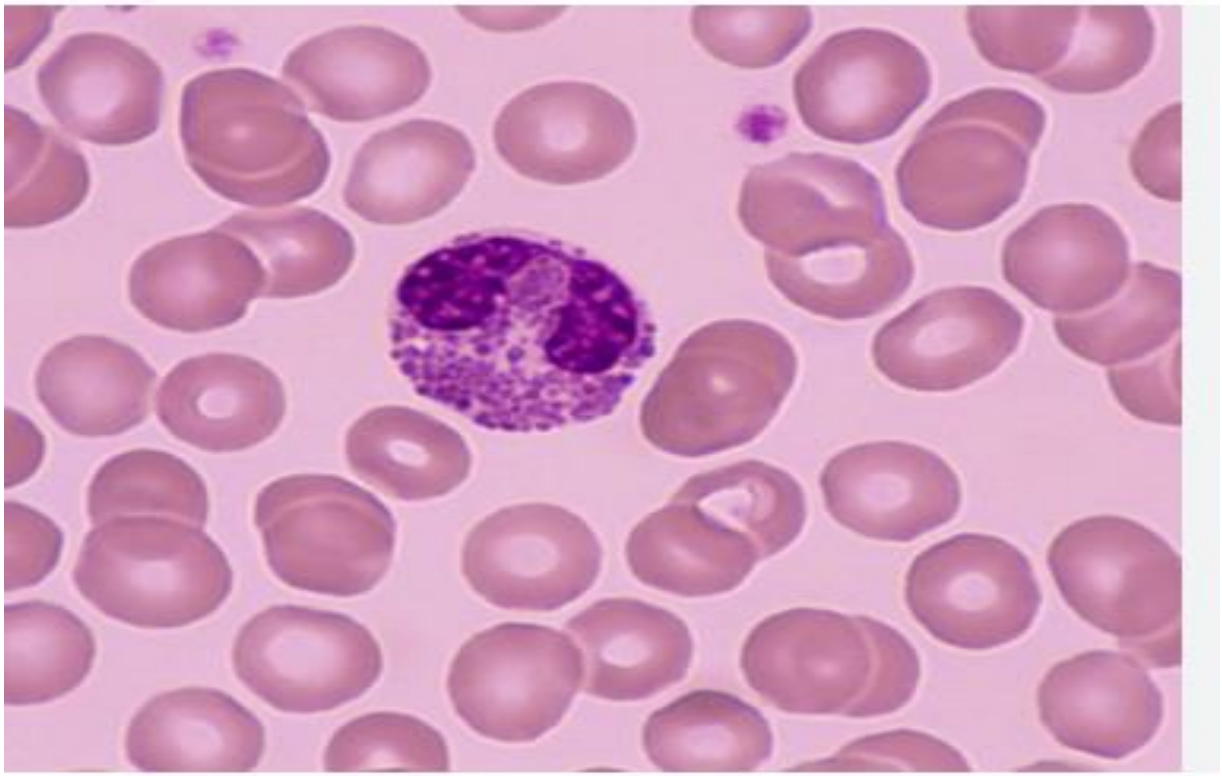
CHARACTERISTIC FEATURES OF RED BLOOD CELLS (Cont.)

- **Production:** Red blood cells are produced in the bone marrow and are regulated by the hormone erythropoietin, which is produced by the kidneys.
- **High density:** Red blood cells are highly dense, which allows them to sink to the bottom of the blood vessels and pass through the narrowest capillaries.

CHARACTERISTIC FEATURES OF RED BLOOD CELLS (Cont.)

- **High concentration of hemoglobin:** Each red blood cell contains about 270 million molecules of hemoglobin, which binds to oxygen in the lungs and releases it in the body's tissues.
- **Lifespan:** Red blood cells have a lifespan of approximately 120 days before they are broken down and recycled by the body.

CONTD….



METHOD FOR DETERMINATION OF RED BLOOD CELLS

Principle:

- The blood is diluted 200 times in a red cell pipette and the cells are counted in the counting chamber. Knowing the dilution employed, their number in undiluted blood can easily be calculated.

CONTD…

- **Apparatus and Material:**
- RBC pipette,
- Improved Neubauer's chamber
- cover slip
- microscope
- disposable blood lancet/pricking needle
- sterile cotton/gauze swabs
- 70% alcohol/methylated spirit
- Hayem's' fluid (RBC diluting fluid)

CONTD…

- **Composition of Hayem's fluid.**

- 1. Sodium chloride (Na Cl) 0.50 g: It provides isotonicity so that the red blood cells remain suspended in diluted blood without changing their shape and size.

- 2. Sodium sulfate (Na,SO4) 2.50 g: It acts as an anticoagulant and as a fixative to preserve their shape and to prevent rouleaux formation.

CONTD…

- **Composition of Hayem's fluid.**

- 3. Mercuric chloride ($HgCl_2$) 0.25 g: It acts as an antifungal and antimicrobial agent and prevents contamination and growth of microorganisms.

- 4. Distilled water 100ml: It is used for dissolving all the above chemicals.

PROCEDURE

- 1. Place about 2 ml of Hayem's fluid in a watch glass.
- 2. Adjust and focus Neubauer's chamber with the cover slip centered on it under low magnification. Adjust the illumination and focus the central 1mm square (RBC square on the counting grid) containing 25 groups of 16 smallest squares each. All these squares will be visible in one field. Do not change the focus or the field.

PROCEDURE

- 3. Move the chamber to your work table for charging it with diluted blood.

- 4. Get a finger prick after cleaning the finger tip. Wipe the first 2 drops of blood and fill the RBC pipette from a fresh drop of blood up to the mark 0.5.

PROCEDURE (Cont.)

5. Suck Hayem's fluid to the mark 101 and mix the contents for 2-3 minutes.

6. Discard first 2-3 drops from the RBC pipette to remove the diluting fluid and place the tip of the RBC pipette at the corner of the cover slip and the diluting fluid will move by capillary action to charge the chamber.

PROCEDURE (Cont.)

- 7. Wait for 3-4 minutes for the cells to settle down, because they cannot be counted when they are moving and changing their positions due to currents in the fluid.

- 8. Counting of red blood cells: Switch over to high magnification (High power lens).

PROCEDURE (Cont.)

- Rules for Counting a. Count the RBCs in 5 small squares i.e. outer 4 corner and 1 central square.
- Thus, the counting would be done in 80 smallest squares, i.e., in 5 blocks of 16 squares each

- Follow Thomas rule during counting i.e. leave lower and left.

PROCEDURE (Cont.)

- **Thomas Rule.**
- It states that cells lying on left and lower lines of square are considered to be outside the square.

- Calculate the number of RBCs/mm' in undiluted blood according to the following:

PROCEDURE (Cont.)

- **Calculation of Size and Volume**
- Size of 1 smallest square
- =1/5x1/4-1/20mm
- Area of 1 smallest square

PROCEDURE (Cont.)

- =1/20x1/20=1/400 mm^2
- Depth of 1 smallest square
- = 1/10 mm

PROCEDURE (Cont.)

Volume of 1 smallest square

- = 1/400 x 1/10 1/4000 mm

- Calculation of Red Blood Cells Count

- Number of Red cells in 80 small squares

- =X Red Blood Cells (RBCs)

PROCEDURE (Cont.)

Number of Red cells in 01 small square

- XRBCS 80
- Number of RBCs in 1mm' in diluted blood
- = X 4000=Y 80
- Dilution factor

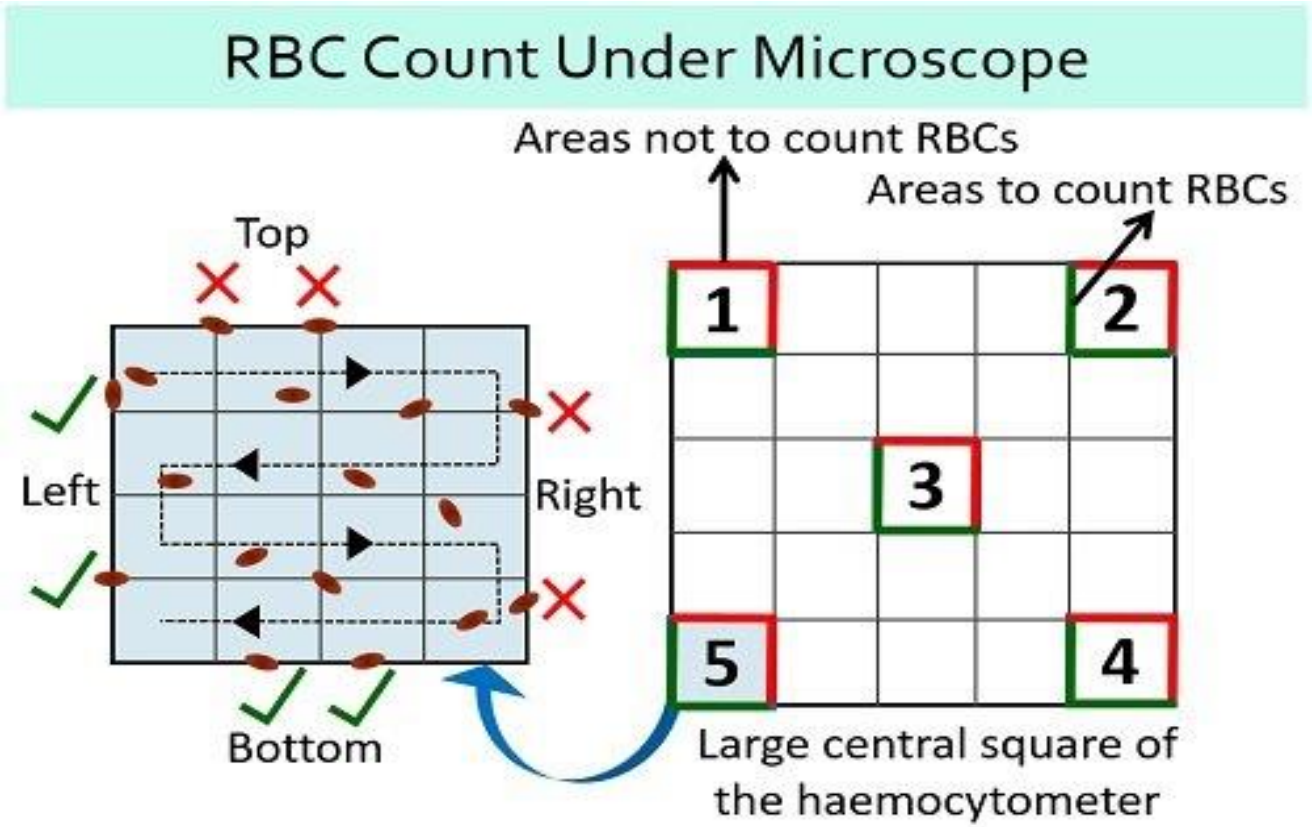
= 200 times

PROCEDURE (Cont.)

Number of RBCs in 1mm' in undiluted blood

- =Yx 200 cells/mm'
- **Calculation of Dilution Factor**
- Dilution with RBC pipette can be 1 in 100 or 1 in 200 depending on whether blood is taken to mark 1.0 or 0.5.
- The Dilution= (Final volume attained (100 parts)/ (Volume of blood taken (0.5 part)

PROCEDURE (Cont.)



RESULTS

- Express your result as = ……. Million/mm3
- Normal Red Blood Cell Count
- The average cell counts and their ranges are;
- Males =5.0 million/mm3 (4.75-6.0 million/mm3) Females=4.5 million/mm3 (4.00-5.5 million/mm3)

QUESTIONS

Question No 1. : When blood is taken to the mark 0.5 and diluted to the mark 101, why is the dilution 1 in 200 and not 2 in 202

Answer:

Dilution of the blood occurs in the bulb, the volume of which is 101 1.0=100. Hence half volume in hundreds gives a dilution of 1 in 200.

QUESTIONS

Question No 2. : Why is the blood diluted 200 times for red cell count?

Answer:

A high dilution is needed because the number of RBCs is very high.

QUESTIONS

Question No 3. : What is the function of bead in the bulb?

Answer:

The bead (red in this case) helps in mixing the contents of bulb thoroughly. It helps in identifying the pipette at a distance. And, thirdly, it tells whether the bulb is dry or not. (If it is not, the bead will not roll freely).

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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