Department of Anesthesia Technique
Title of the lecture:

Red blood cells count

Lec – 7

By

Msc. Amaal Sahib
Msc. Faten Kareem
Dr. Zahraa Tariq
Dr. Duaa Hamza
Msc. Ola Abdulla

Red Blood Cells Shape

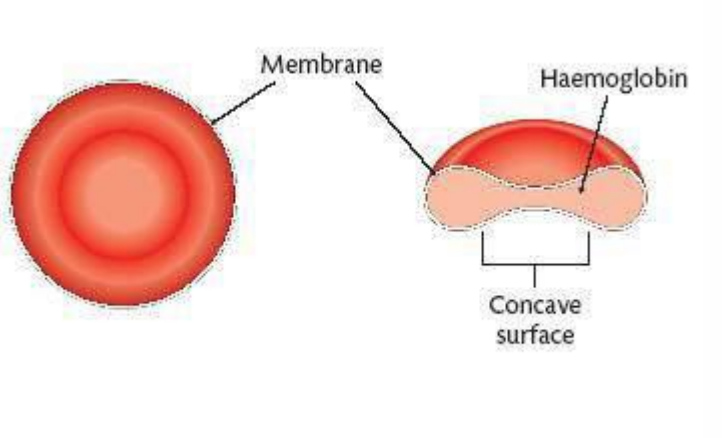
- Red blood cells (RBCs) are the round shape, biconcave discs, present in the blood that helps in the transport of gases throughout the body.

- The biconcave shape helps the RBCs in rendering the red cells quite flexible so that they can easily pass through the capillaries. On an average, the size of the Red Blood

- Cells (RBCs) is 7.2 – 7.4 mm (microns).

- The mature RBCs are non-nucleated cells with an Iron-containing pigment known as Hemoglobin which helps in the transport of oxygen from the lungs to tissues and carbon dioxide from tissues back to the lungs for excretion.

- The Average lifespan of Red Blood Cells (RBCs) is 100 – 120 days.



RBCs count

- A RBC count is a blood test that tells how many red blood cells (RBCs) you have

The RBC measurement is used to help diagnose red blood cell disorders, such as anemia.

- **The general the range is as follows:**
- **Male: 4.7 to 6.1 million cells per microliter (cells/mcL)**
- **Female: 4.2 to 5.4 million cells/mcL**

Red blood cells disorders

Lower-than-normal numbers of RBCs may be due to:

1-Anemia

2-Bone marrow failure (for example, from radiation, toxins, or tumor)

3-Erythropoietin deficiency (kidney disease)

4- Hemolysis (RBC destruction)

5- Hemorrhage (bleeding)

6- Leukemia

7- Malnutrition

8- Multiple myeloma

9- Nutritional deficiencies of: * Iron * Copper * Folate * Vitamin B-12 * Vitamin B-6

10- Over hydration

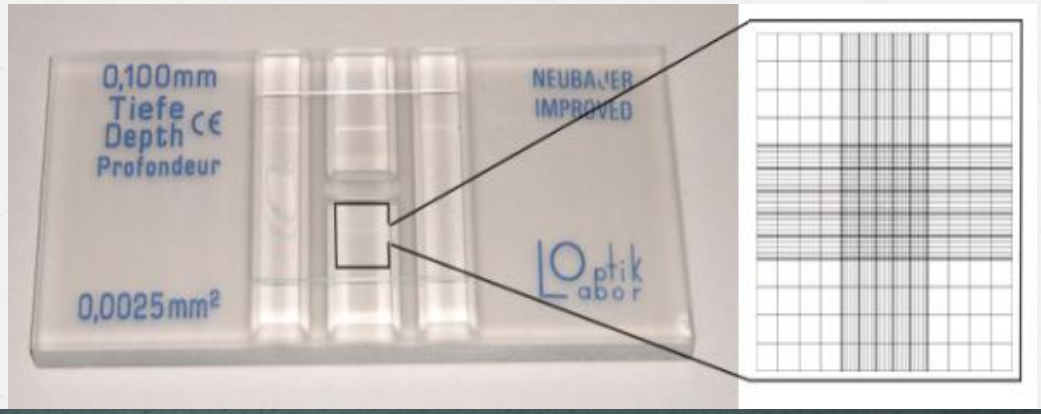
11- Pregnancy

Equipment and reagents

1- Neubauer's Chamber or Haemocytometer

It is a specialized thick glass slide used to count the eukaryotic cell suspension. Haemocytometer has a size of 30 X 70 X 4 mm. Its central portion is ruled, where the cell counting is performed.

- Nine large squares have an overall width of 3 mm.
- Twenty-five medium squares in each large square have a width of 0.25 mm.
- Sixteen small squares in each medium square have a width of 0.05 mm.



2- RBC Pipette

It is commonly used to dilute the blood sample with the RBC diluting fluid.

3-Micropipette

4-RBC diluting fluid

5-Blood sample (Capillary blood

or EDTA anticoagulated specimen)

6-Gauze piece or Cotton

7- Coverslip

8-Microscope

Procedure

RBC-Diluting Fluid

two types of RBC Diluting fluid are commonly used in Laboratories

1- Hayem's RBC Diluting fluid

COMPONENTS	QUANTITY
Mercuric Chloride	0.25 grams
Sodium sulfate	2.5 grams
Sodium chloride	0.5 grams
Distilled water	100 ml

2- Formalin Citrate diluting fluid

COMPONENTS	QUANTITY
Trisodium citrate	3 grams
Formalin	1 ml
Distilled water	99 ml

Procedure of the Total Red Blood Cell (RBC) Count by Microdilution Method

1- Fill the RBC pipette up to the 0.5 mark with the blood specimen and wipe out the pipette externally to avoid false high results.

2- Fill the same pipette with the RBC diluting fluid (preferably Hayem's Fluid) up to the mark 101.

3- Be cautious that there should be no air bubble in the pipette bulb.

4- Mix the Blood and Diluting fluid in the pipette by rotating the pipette (horizontally) between your palms.

5- Take out the Neubauer's chamber / Hemocytometer from its case and clean it using a swab or gauze piece. Similarly, clean out the cover glass and place it over the grooved area of Hemocytometer.

6- Now, put the RBC pipette, mix the solution present in it again and then discard 1-2 drops from the pipette before charging the chamber.

7- Gently press the rubber tube of the RBC pipette, so that the next drop of fluid is in hanging position.

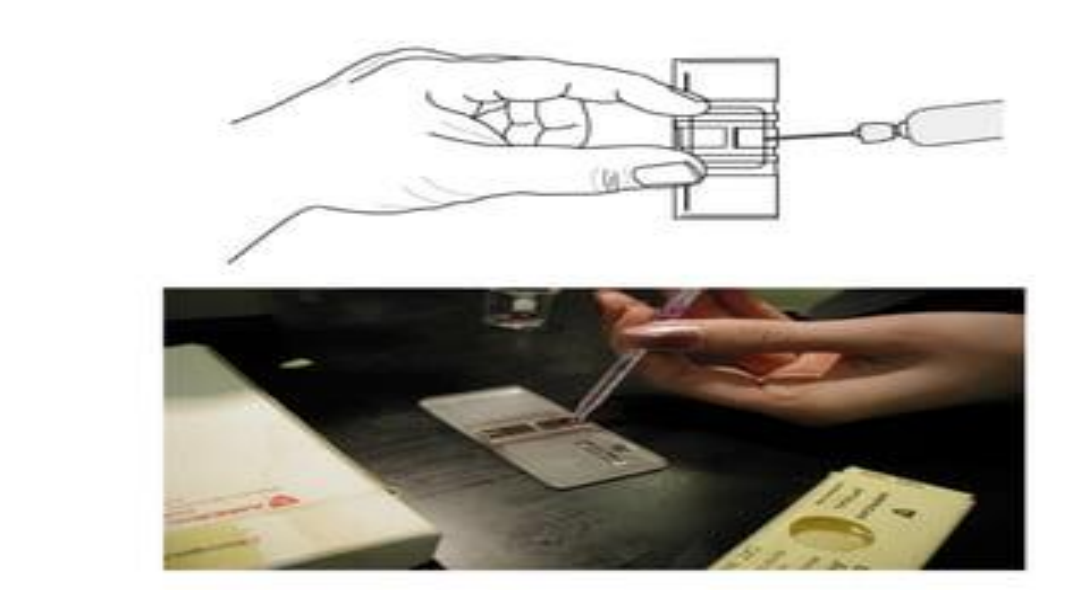
8- Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

9- Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.

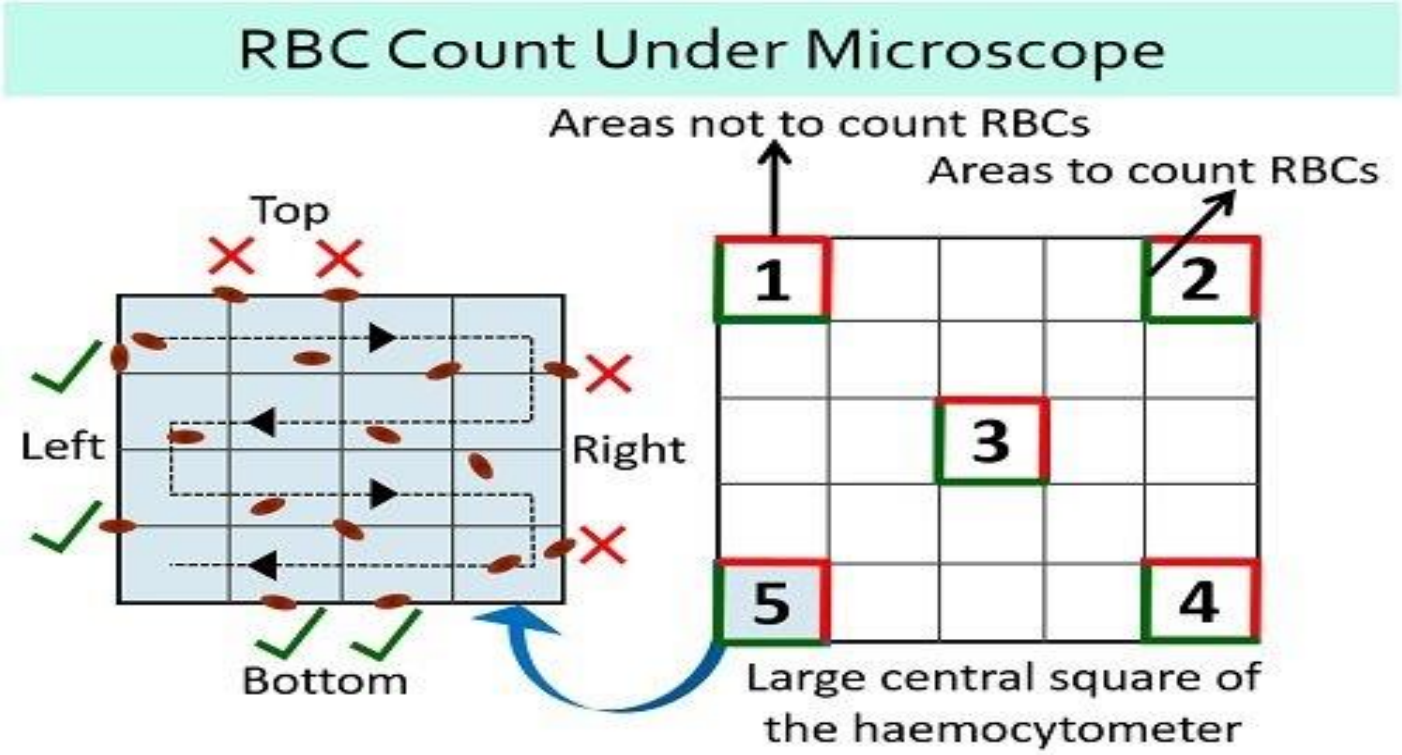
10- After charging, wait for 3-5 min so that the cells settle down in the chamber & then focus the chamber under the microscope to calculate Red Cells.

RBC Count under Microscope

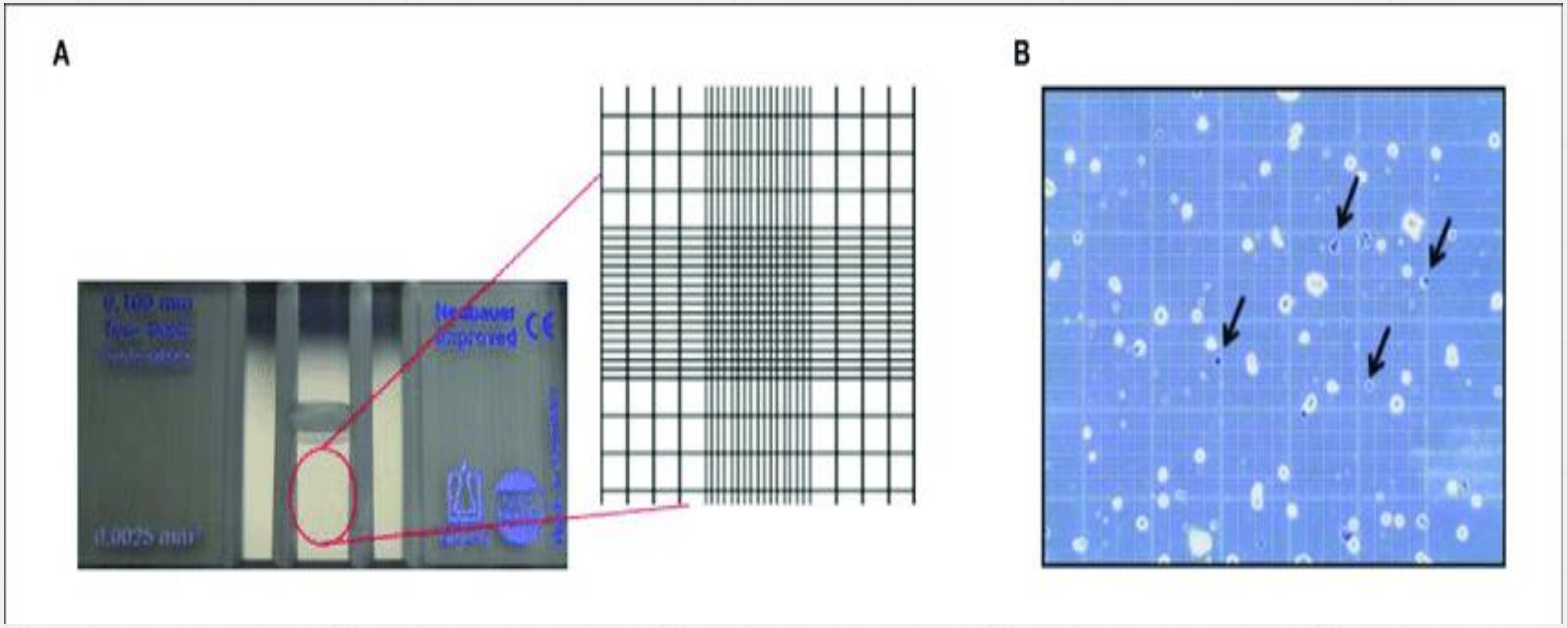
First, focus the rulings of the haemocytometer slide using a 10X objective lens. Using coarse and fine adjustment knobs, focus on the five squares of the large central square to count the number of red blood cells under the 40X objective.



A diagram represents the pattern to count RBCs in all the five medium squares of a large central square. As already discussed, each medium square possesses 16 small squares. We need to manually count the number of RBCs in five medium squares via hand tally



In each square, you need to count the red blood cells located within the square. The red lines in the upper and right corners indicate the areas not to count RBCs, whereas green lines indicate the areas to count the RBCs.

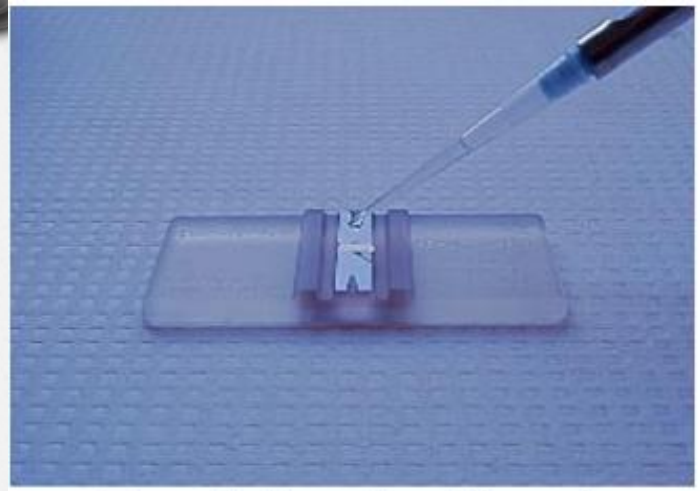


Calculation of RBCs

Total RBC Count = N × Dilution / Area × Depth

N × 200 (or 100 as the dilution is made) / (1/5 × 0.1)

Total RBC count = N × 10,000 / mm^3



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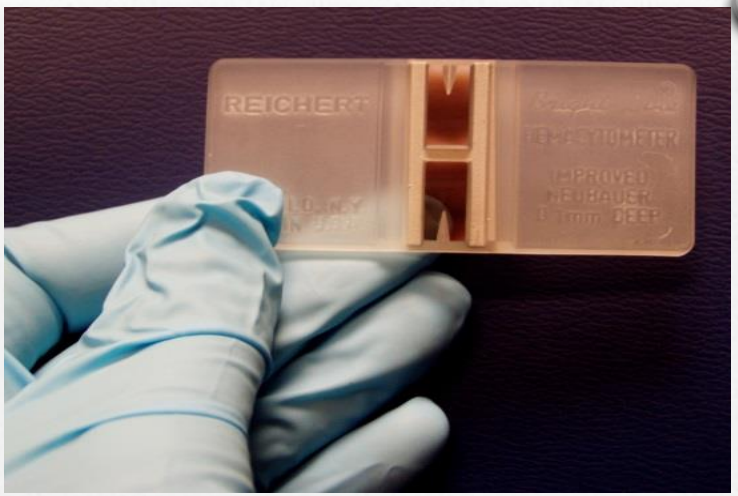

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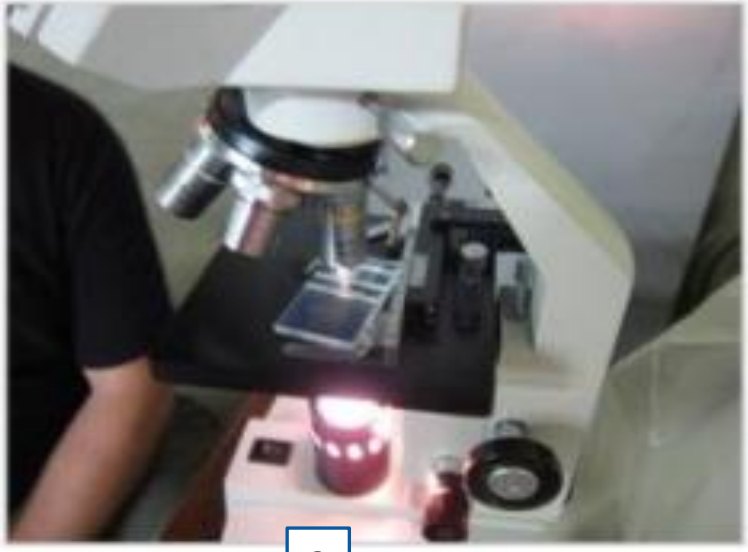

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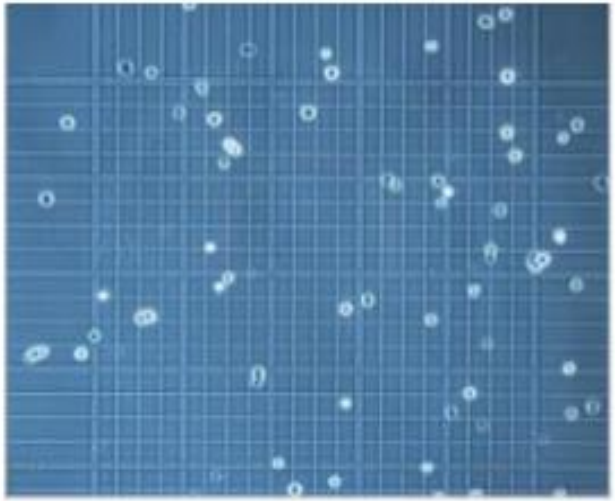

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