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HEMATOLOGY PRACTICALS

TOTAL RED BLOOD CELL (RBC) COUNT USING HEMOCYTOMETER / NEUBAUER's CHAMBER (MICRO DILUTION & MACRO DILUTION METHOD)

April 6, 2018 / SAHIL BATRA

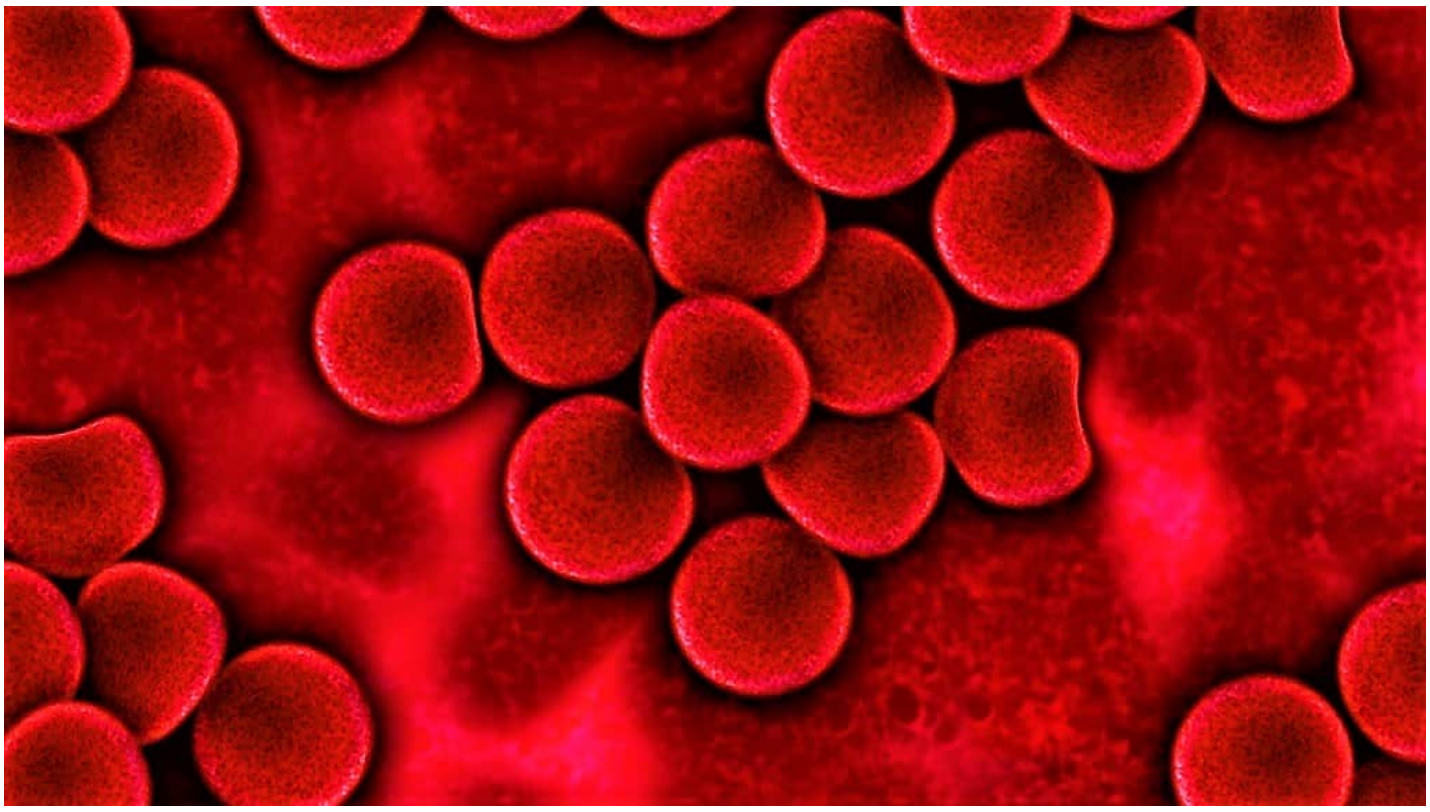
A BRIEF INTRODUCTION TO RED BLOOD CELL

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.

Check out the Composition of Blood

THE AIM / PURPOSE OF PERFORMING TOTAL RBC COUNT

The purpose of performing Total Red Blood cell count is to know whether or not you are suffering from Erythrocytosis or Polycythemia (i.e. the increase in the no. of Red Blood Cells to more than 6.5 million/mm^3) or Erythrocytopenia or Erythropenia (i.e. the Decrease in the no. of Red Blood Cells to less than 3.5 million/mm^3).



RED BLOOD CELLS

PRINCIPLE OF TOTAL RBC COUNT USING HEMOCYTOMETER

Very large numbers of Red Blood Cells are present in the Blood Specimen. Practically, counting this amount of Red cells directly under the microscope is highly impossible. So, the Red Blood cells are counted by using a special type of chamber, designed for the counting of blood cells in the specimen, known as Hemocytometer or Neubauer's chamber.

For this, the blood specimen is diluted (usually in 1:200 ratio) with the help of RBC diluting fluid (commonly the Hayem's Fluid) which preserve and fix the Red blood cells. The Hayem's fluid is isotonic to the Red blood cells and does not cause any damage to it. The Normal Saline solutions can also be used for this but it causes the slight creation of red blood cells and allows rouleaux formation which may cause the errors in results.

After diluting the specimen, the content is charged on Hemocytometer / Neubauer's chamber and the cells are counted in the areas specific for RBC count.

Nowadays, two types of RBC Diluting fluid are commonly used in Laboratories –

- Hayem's RBC Diluting fluid
- Formalin Citrate diluting fluid

The composition of Hayem's diluting Fluid

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

The Final pH of the solution (at 25°C) varies from 5.8 – 6.0 which depends on the composition and companies who manufacture it.

The above Composition is based on HIMEDIA Hayem's diluting fluid protocol which you can check here.

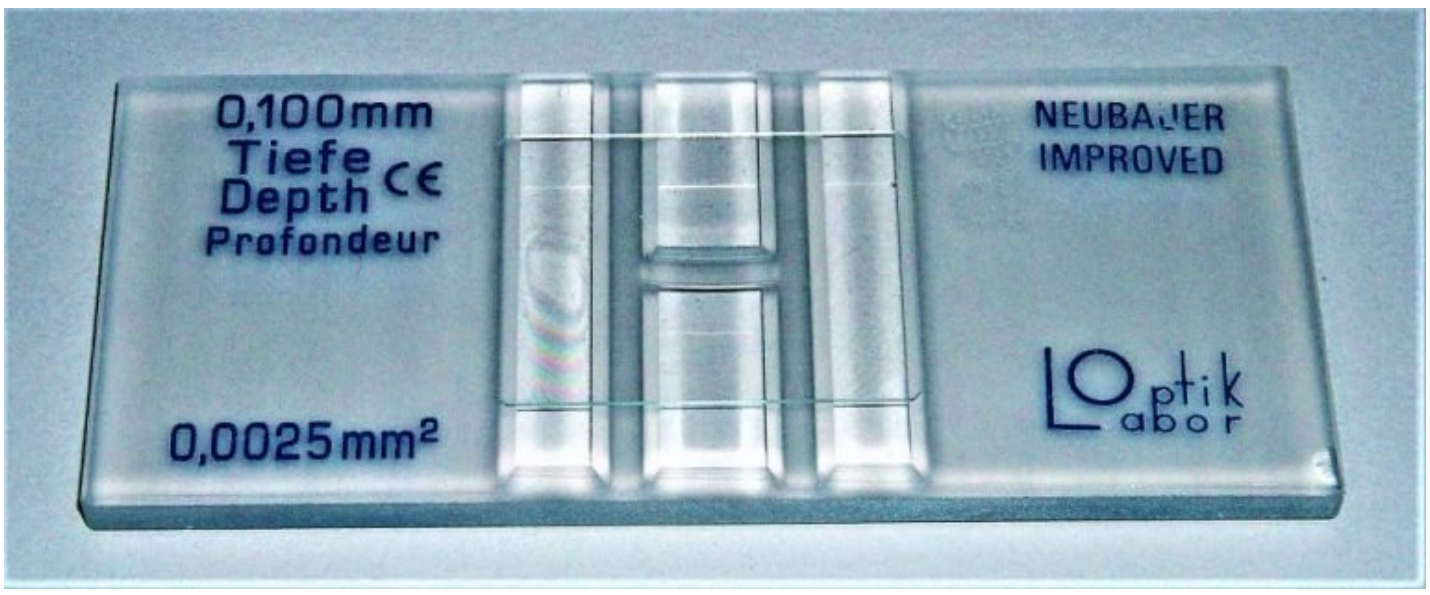
The composition of Formalin Citrate diluting fluid

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

This diluting fluid is commonly used because it is cheaper than the Hayem's fluid. However, Hayem's diluting fluid gives the better results.

Hemocytometer / Neubauer's Chamber –

This is a special type of glass chamber that is used for the cell counting, especially for Blood cells. Nowadays, most commonly Improved Neubauer's Chamber is used and in some laboratories, other types of chambers are also employed like Burkers chamber, Levy's chamber and Fusch – Rosenthal chamber etc.

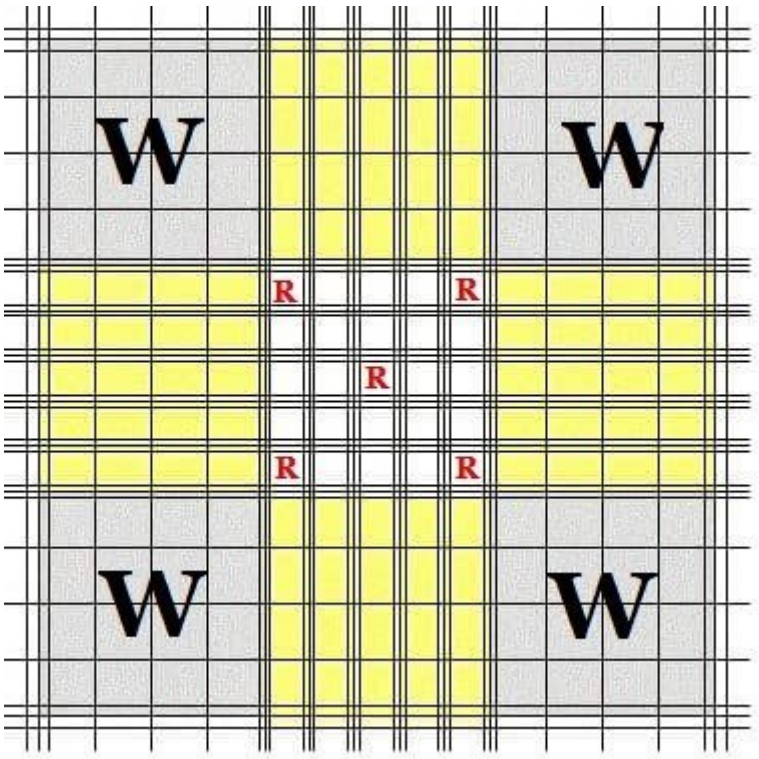


HEMOCYTOMETER A.K.A NEUBAUER's CHAMBER

The Neubauer's Chamber has ruled the area of total 9 square mm and the depth is 0.1 mm as when the coverslip is placed on the surface of the counting chamber, the space between the bottom of the cover glass and the base of grooved area measures 0.1 mm in depth.

The central 1 square is highly ruled which is divided into 25 squares. Each square of the Central square is further subdivided into 16 small squares.

For RBC count the cells are counted in the 5 squares of the Central square as 4 Corner squares of the Central square (divided into 25 squares) and 1 central square of the Larger Central Square (divided into 25 squares).



R – RBC AREAS W – WBC AREAS

Each square of the Central Square (divided into 25 squares) contains 16 small squares so the total no. of the area to be counted for RBC Count –

16 × 5 = 80 small squares

Two Method has been developed for the Manual Estimation of Total Red Blood Cell Count Using Hemocytometer / Neubauer's chamber –

- **Microdilution Method**
- **Macrodilution Method**

Here, I'll explain both the methods but the Microdilution method is not preferred nowadays due to the use of Mouth pipettes. So let's start with Microdilution method and then we'll move to Macrodilution method….

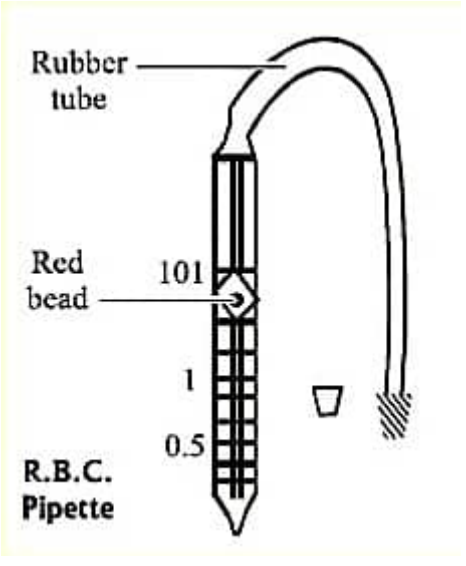
MICRODILUTION METHOD FOR THE ESTIMATION OF TOTAL RBCs USING HEMOCYTOMETER

Materials Required for the Total Red Blood Cell (RBC) Count by Microdilution Method –

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- RBC diluting fluid (preferably Hayem's fluid)
- Gauze piece or Cotton
- RBC pipette
- Hemocytometer a.k.a. Neubauer's Chamber
- Coverslip
- Microscope

A Brief Introduction to RBC Pipette

RBC pipette is a graduated pipette that gives the dilution of 1:100 and 1:200. It has two markings at the bottom as 0.5 and 1 and the top of the pipette is marked 101. It has a round shape bulb which contains the Red bead to mix the blood specimen and the diluting fluid. On the top, a rubber tube is attached to the pipette for sucking the blood specimen and diluting fluid.



RBC PIPETTE

When blood is sucked up to 0.5 mark and the diluting fluid up to 101 marks, gives the 1:200 dilution of Blood: Diluting fluid and When the Blood is sucked up to 1 mark and the diluting fluid up to 101, gives the 1:100 dilution of Blood: Diluting fluid which is commonly used in anemic patients. After sucking the Specimen & Diluting fluid, the content is gently mixed by rotating the pipette on its long axis to ensure thorough mixing of blood and diluting fluid.

Note: *Nowadays Mouth pipetting is banned in most of the laboratories due to the high risk of getting infected with highly infected specimens of the patients. So instead of Microdilution method, the Macrodilution methods are employed in Laboratories…..*

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

⇒ 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.

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

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

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

⇒ 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.

Note: *Here a special type of cover glass is used which is 0.4 mm thick with very smooth surface and even thickness so that the space between the grooved area of the chamber and cover glass is exactly 0.1 mm.*

⇒ 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.

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

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

⇒ 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.

⇒ 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.

MACRODILUTION METHOD FOR THE ESTIMATION OF TOTAL RBCs USING HEMOCYTOMETER

Materials Required for the Total Red Blood Cell (RBC) Count by Macrodilution Method

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- RBC diluting fluid (preferably Hayem's fluid)
- Hb pipette or Micropipette (0.02 ml or 20 *µl*)
- Hemocytometer / Neubauer's Chamber
- Gauze piece or Cotton swab
- Graduated Pipette (5 ml)
- Test tubes
- Cover Slip

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

⇒ Take 3.98 ml of RBC diluting fluid in a Clean, Dry and Grease free Test tube.

Note: *If you don't have variable pipette in the lab which can measure 3.98ml or 3980 µl of Diluting fluid then Take 4 ml of Diluting fluid with the help of 5ml Graduated pipette in the test tube and discard 20 µl of fluid using a micropipette or RBC pipette.*

⇒ Now add 0.02 ml or 20 *µl* of Blood Specimen to the tube containing diluting fluid with the help of micropipette or RBC pipette.

⇒ Mix well for few minutes and ready your Hemocytometer / Neubauer's Chamber.

⇒ 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.

Note: *Here a special type of cover glass is used which is 0.4 mm thick with very smooth surface and even thickness so that the space between the grooved area of the chamber and cover glass is exactly 0.1 mm.*

⇒ Now, take out the RBC pipette and fill it with the Diluted Specimen, mix the solution well and then discard 1-2 drops from the pipette before charging the chamber.

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

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

⇒ 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.

Using Micropipette instead of RBC pipette for charging the Hemocytometer

⇒ You can also use a micropipette instead of RBC pipette for charging the Hemocytometer. So, with a micropipette, carefully draw up around 20 *µl* of the diluted specimen. Press the knob of the pipette to make a hanging drop at the tip of the micropipette.

⇒ Now gently place the pipette tip against the edge of the cover glass and if required slowly expel the more liquid until the counting chamber is full. This process occurs by Capillary action, but care should be taken not to overfill the chamber. A volume of 10 *µl* is sufficient to fill out the one counting chamber.

⇒ After charging, wait for 3-5 min so that the cells settle down in the chamber.

COUNTING THE RED BLOOD CELLS UNDER MICROSCOPE

⇒ Focus the ruling using the 10x Objective lens and then Count the RBCs in 5 small squares of the central square as described above, using the 40x Objective lens.

⇒ Count the cells which are lying on the right and lower lines of the 5 small squares but not the opposite line. In case of marginal cells, count the cells on 'L' line that is either on Right and Lower lines or Left and Upper lines.

CALCULATIONS FOR THE TOTAL RBC COUNT USING HEMOCYTOMETER

⇒ After counting the cells under the microscope, we know the No. of RBC in 5 squares of the central square. Let's consider it as 'N' no. of cells.

⇒ Now, the volume of the fluid inside the chamber is the product of Area and depth of the Hemocytometer / Neubauer's chamber.

⇒ The central area is the 1 sq. mm which is divided into 25 parts so the area is

25 squares = 1 sq. mm

⇒ Out of these 25 squares, the RBCs are counted in 5 squares. So the Area of 5 small squares is

5/25 i.e. 1/5

⇒ The depth of the Hemocytometer is 0.1 mm as described above in a short description of Hemocytometer.

⇒ Now Apply the Following formula to get the Total Red Blood Cell Count –

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

Using the Above formula we can calculate the Total No. of Red Blood Cells present in the Blood Specimen.

NORMAL VALUES OF RED BLOOD CELLS

In Males – 4.8-5.5 million/mm^3

In Females – 4.5-5 million/mm^3

PRECAUTIONS TO BE TAKEN WHILE PERFORMING TOTAL RBC COUNT BY HEMOCYTOMETER

⇒ Use of Mouth pipettes (RBC pipette) is banned in many countries. However, in case you have to use it, be cautious that you should not intake the diluting fluid or Specimen.

⇒ Accurately measure the amount of specimen and Diluting Fluid to avoid any error in the results.

⇒ In case you are performing this test by Microdilution method, mix the specimen and diluting fluid appropriately by gently rotating in between your palms.

⇒ Carefully charge the Hemocytometer or Neubauer's chamber that it should not be overcharged and do not contain any air bubble in it.

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Hi, I'm the Founder and Developer of Paramedics World, a blog truly devoted to Paramedics. I am a Medical Lab Tech, a Web Developer and Bibliophiliac. My greatest hobby is to teach and motivate other peoples to do whatever they wanna do in life.

Tags: CALCULATIONS FOR TOTAL RBC COUNT, CALCULATIONS FOR TOTAL RED BLOOD CELL COUNT, HAYEM's FLUID, HEMATOLOGY PRACTICALS, HOW TO PERFORM TOTAL RED CELL COUNT IN LABORATORY, MACRODILUTION METHOD OF TOTAL RBC COUNT, MICRODILUTION METHOD OF TOTAL RBC COUNT, NEUBAUER CHAMBER, NEUBAUER's CHAMBER, PRINCIPLE OF RED BLOOD CELL COUNT USING HEMOCYTOMETER, RBC DILUTING FLUID, SIGNIFICANCE OF TOTAL RBC COUNT, TOTAL RED BLOOD CELL COUNT BY HEMOCYTOMETER, TOTAL RED CELL COUNT BY HEMOCYTOMETER, TOTAL RED CELL COUNT IN LABORATORY, TOTAL RED CELL COUNT USING HEMOCYTOMETER, TOTAL RED CELL COUNT USING NEUBAUER's CHAMBER, WHAT IS HEMOCYTOMETER, WHAT IS RBC DILUTING FLUID, WHAT IS THE COMPOSITION OF HAYEM's FLUID, WHAT IS THE PURPOSE OF TOTAL RED BLOOD CELL COUNT