Manual Blood cell counts

Counting chamber Hemocytometer

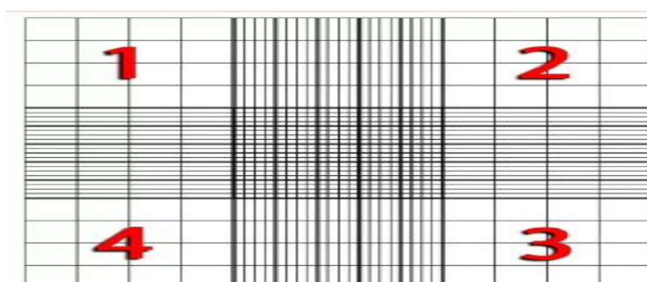
An instrument for visual counting of the number of cells in a blood sample or other fluid under a microscope. Hemocytometer; Hemo, for blood; cyto, for cell; meter, for measuring. So altogether: measuring blood cells.

How does a hemocytometer work?

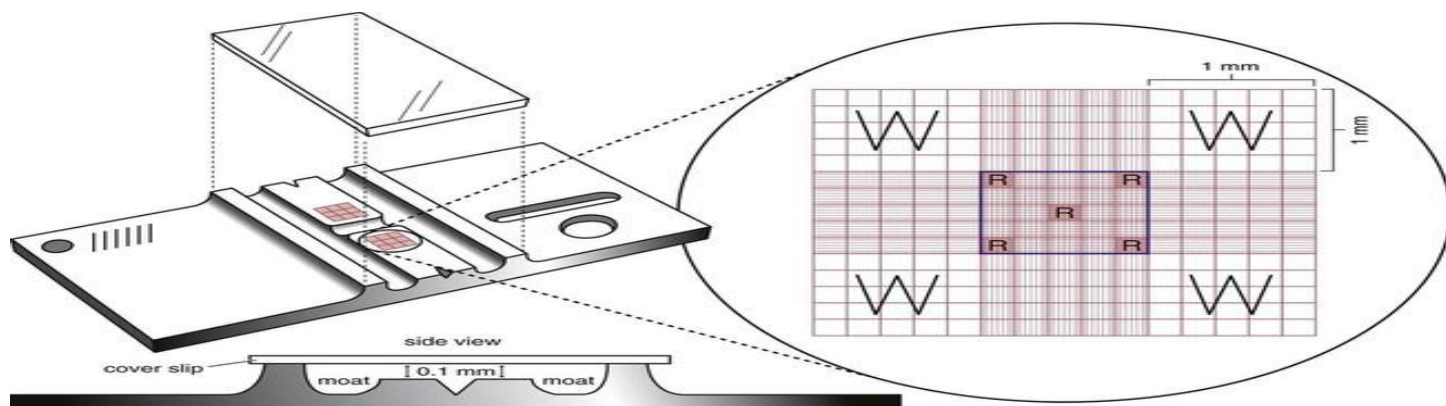
This big square has other little squares inside, following the pattern below:

Each of the nine squares inside the big square is equal in surface. In turn, each of the sixteen little squares inside each of the four corner squares is equal in surface.

We count the White Blood Cells in the 4 squares at the corners (i.e. the numbered squares in the next image is at the center)



While we **Red Blood Cells** and **platelets** count in 5 square as those marked in the below image:



Principle of the test:-

- Whole blood is diluted 1 in 20 in an acidic reagent which hemolysis the red cells and leave the white cells to be counted.
- Whole blood is diluted 1 in 20 in an ammonium oxalate reagent which hemolysis the red cells and leave the platelets to be counted.
- Whole blood is diluted 1 in 200 in an isotonic solution which avoid red cells RBCs rupture due to osmotic pressure.

Tools and reagents:-

1- Counting chamber (Hemocytometer).
2- Counting chamber cover glasses.
3- Micropipette and capillaries.

Procedure:-

Blood cell counts is performed in 4 stages;

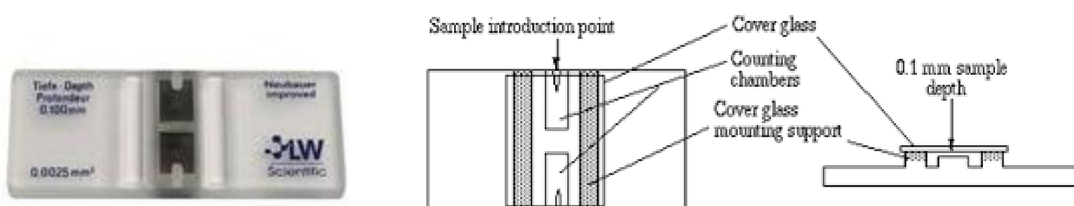
A. Diluting
B. Hemocytometer loading
C. Counting
D. Calculating.

Test	WBC	RBC	Platelet
Sample	EDTA anticoagulated blood or capillary blood	Whole blood	EDTA anticoagulated blood.
Note	Heparin or sodium citrate anticoagulated blood must not be used. The count should be performed within 6 hr (blood should not be refrigerated).	——	Capillary blood should not be used because platelets clumps during the collected of blood.
Diluting fluid	Acetic acid 2% v/v & Gention violet 1% w/v	Isotonic solution	Ammonium oxalate reagent
Diluting ratio	diluted 1 in 20	diluted 1 in 200	diluted 1 in 20

Calculation	Summation of cells counted by the hemocytometer × 50	Summation of cells counted by the hemocytometer ×1000	Summation of cells counted by the hemocytometer ×10000
Unit	WBCs/µl or WBCs / mm^3	RBCs/µl or RBCs / mm^3	PLTs/µl or PLTs / mm^3

B- Hemocytometer loading

From the picture, the hemocytometer consists of 2 counting chambers and hence 2 sites for counting and you can see the 2 sites for applying sample (V shaped).

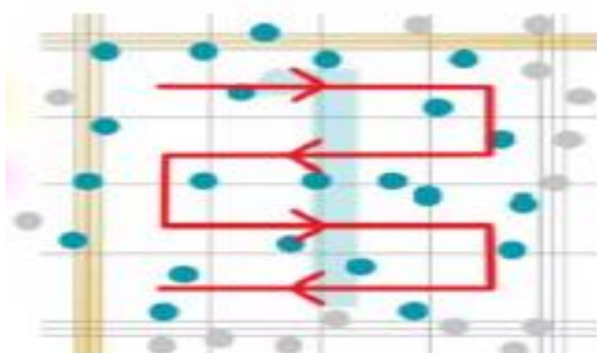


1. Clean the cover glass and the counting chambers with alcohol swap to make moisture.
2. Slide the cover glass into position over the grid areas.
3. Re-mix the diluted blood sample.
4. Using a capillary, Pasteur pipette, or plastic bulb pipette held at an angle of about 45 , pipette the diluted blood to load one of the grids of the chamber with the sample, taking care not to overfill the area.

➢ Important: The chamber must be refilled if the sample overfills into the channel beyond the grid or an air bubble forms in the grid area.

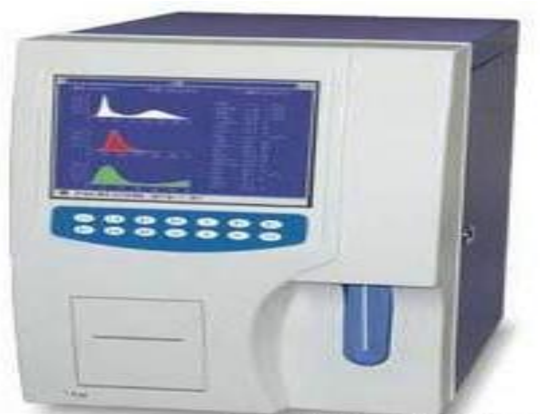
C- Counting Chamber adjustment:

Count the cells in all the small squares in a zigzag manner.



Hematology analyzer technology

The three main physical technologies used in hematology analyzers are: electrical impedance, flow cytometry, and fluorescent flow cytometry. These are used in combination with chemical reagents that lyse or alter blood cells to extend the measurable parameters. For example, electrical impedance can differentiate red blood cells (RBCs), WBCs, and platelets by volume. Adding a nucleating agent that shrinks lymphocytes more than other WBCs makes it possible to differentiate lymphocytes by volume.



Electrical impedance

The traditional method for counting cells is electrical impedance, also known as the Coulter Principle. It is used in almost every hematology analyzer.

Whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass through at a time. The impedance changes as a cell passes through. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume.

Impedance analysis returns CBCs and three-part WBC differentials (granulocytes, lymphocytes, and monocytes) but cannot distinguish between the similarly sized granular leukocytes: eosinophils, basophils, and neutrophils.

Counting rates of up to 10,000 cells per second can be achieved and a typical impedance analysis can be carried out in less than a minute.

Flow cytometry

Laser flow cytometry is more expensive than impedance analysis, due to the requirement for expensive reagents, but returns detailed information about the morphology of blood cells. It is an excellent method for determining five-part WBC differentials.

A single-cell stream passes through a laser beam. The absorbance is measured, and the scattered light is measured at multiple angles to determine the cell's granularity, diameter, and inner

complexity. These are the same cell morphology characteristics that can be determined manually from a slide.

Fluorescent flow cytometry

Adding fluorescent reagents extends the use of flow cytometry to measure specific cell populations. Fluorescent dyes reveal the nucleus-plasma ratio of each stained cell. It is useful for the analysis of platelets, nucleated RBCs, and reticulocytes.

Proprietary technologies

Manufacturers combine these three technologies with innovative uses of reagents, hydrofluidics, and data analysis tools to produce proprietary methods, each of which has strengths in terms of accuracy, speed, or breadth of parameters.