BLOOD CELL COUNTERS

Changes in the normal functioning of an organism are often accompanied by changes in the blood cell count. Therefore, the determination of the number and size of blood cells per unit volume often provides valuable information for accurate diagnosis.

The blood constitutes 5-10% of the total body weight and in an average adult, it amounts to 5-6 litres. Blood consists of corpuscles suspended in a fluid called plasma in the proportion of 45 parts of corpuscles to 55 parts of plasma.

The majority of the corpuscles in blood are red blood cells others being white blood cells and platelets, Blood cells are divided into groups according to their form and function as shown in the table.

Blood cell types	Number of cell in mm^3	Mean cell volume (MCV) in μm^3
1. Erythrocytes	(4.8 – 5.5) I x 1 x 10^4	90
2. Leucocytes	5000-10000	-
a) Neutrophills b) Lymphocytes c) Eosinophills d) Basophills e) Monocytes	2000-7500 1500-4000 40-400 10-100 200-800	450 250 450 450 600
3. Thrombocytes	1.5 x 10^5 – 4 x 10^5	8

Erythrocytes (Red blood cells)

Red blood cells have the form of a bi-concave disc with a mean diameter of about 7.5µ and the thickness of about 1.7µ. The mean surface area of the cell is about 134μm^2. There are about 5.5 million of them in every cubic millimeter of blood in men and nearly 5 million in women.

Responsibility

The erythrocytes have no nucleus. They are responsible for carrying oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

Leucocytes (White blood cells)

Leucocytes are spherical cells having a nucleus. There are normally 500-10000 white cells per cubic mm of blood, but their number varies during the day. They live for seven to fourteen days and there is a rapid turnover. With constant destruction and replacement.

Responsibility

Leucocytes are responsible to form the defence mechanism of the body against infection.

Thrombocytes

Platelets are usually tiny, round, oblong or irregularly shaped cells of the blood with an average diameter of approximately 2μ. There are usually 250000-750000 platelets in every cubic mm of blood.

Responsibility

They play an important role in the blood coagulation process.

Calculation of size of cells:

i) Mean cell volume (MCV)

It is calculated from the PCV, and the number of red cells present per litre of blood. For example, if PCV is 0.45, i.e., 1 litre of blood contains 0.45 litres of red cells and if there are $5x10^{12}$ red cells per litre, then

Mean volume of the cell = $\frac{0.45}{5\times10^{12}} = 90 f/1$

$\because f/1$ = Femolitres

Normal mean red cell volume is $86 \pm 10 f/1$

ii) Mean cell haemoglobin (MCH)

It is calculated from the Hb and red cell count. For example, If there are 15g of Hb per decilitre (dl) of blood, there will be 150gram Hb per litre of blood and number of red cells is 5×10^{12} per litre, then

$$\text{MCH} = \frac{150}{5\times10^{12}} = 30 \text{ picogram (pg)}$$

Normal mean cell haemoglobin is 29.5 ± 2.5 pg.

iii) Mean cell haemoglobin concentration (MCHC)

It can be calculated if PCV and Hb per dl are known. For example,

If PCV is 0.45 and Hb is 15 pg per dl

then, MCHC = $\frac{15}{0.45}$ g/dl

= 33.3 g/dl

iv)Mean platelet volume (MPV)

It is the ratio of the integrated platelet volume to the platelet count and is expressed in femolitres.

v) Plateletcrit (PCT)

It is the percentage of the total specimen volume occupied by the platelets.

$$PCT\% = \frac{MPV(fl)\ X\ PLT\ X\ (10^9/1)}{10}$$

vi) Red Cell Distribution Width (RDW)

It is a numerical expression of the width of the size distribution of red cells. It is derived by analog computation.

$$\text{RDW index} = \frac{(20^{th} - 80^{th})\ \text{percentile volume}}{(20^{th} + 80^{th})\ \text{percentile volume}} \times 100 \times K$$

K – constant, the calibration factor to produce a result of 10 for a normal population

vii) Platelet Distribution Width (PDW)

This index is related to the size range covered by those platelets lying between the sixteenth and eighty fourth percentile.

Methods of cell counting:

i) Microscopic Method

The most common and routinely applied method of counting blood cells even today, particularly in small laboratories is the microscopic method in which the diluted sample is visually examined, and the cells counted. It is commonly known as the counting chamber technique; it suffers from several common drawbacks.

ii) Automatic Optical Method

This method is based on collecting scattered light from the blood cells and converting it into electrical pulses for counting. A sample of dilute blood is taken in a glass container. It is drawn through a counting chamber in which the blood stream is reduced in cross-section by a concentric high velocity liquid sheath.

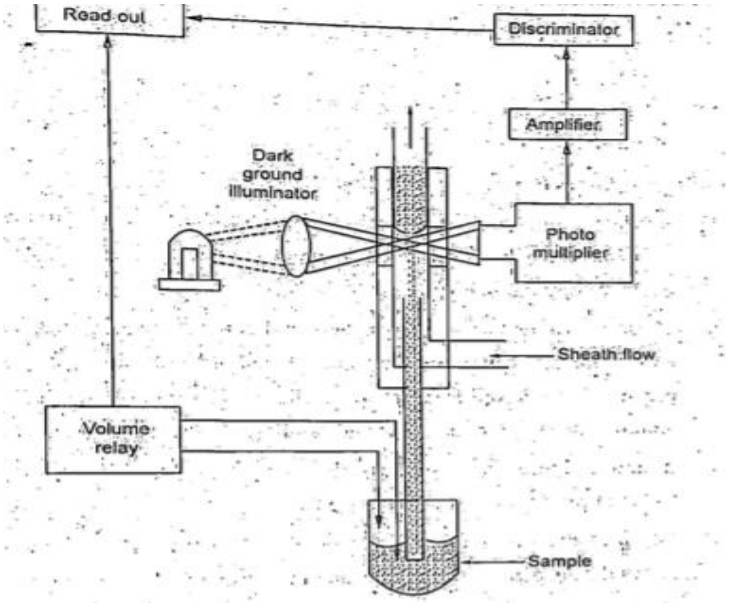

Fig: Optical method of counting cells

A sample optical system provides a dark field illuminated zone on the stream and the light scattered in the forward direction is collected on the cathode of a photomultiplier tube. Pulses are produced in the photomultiplier tube corresponding to each cell.

The discriminator provides pulses of equal amplitude, which are used to drive a digital display. Instruments based on this technique take about 30S for completing the count. An accuracy of 2% is attainable. The instruments require about 1 ml of blood sample.

iii) Coulter Counters (Conductivity Method)

A wide range of particle counting instruments based on the coulter principle have been designed to meet a wide variety of needs in the haemotology laboratory.

These instruments range from the small counters used primarily for red and white cell counts in very small hospitals and clinics, to the multiparameter microprocessor controlled instrument featuring fully automatic diluting of samples and printing of results.

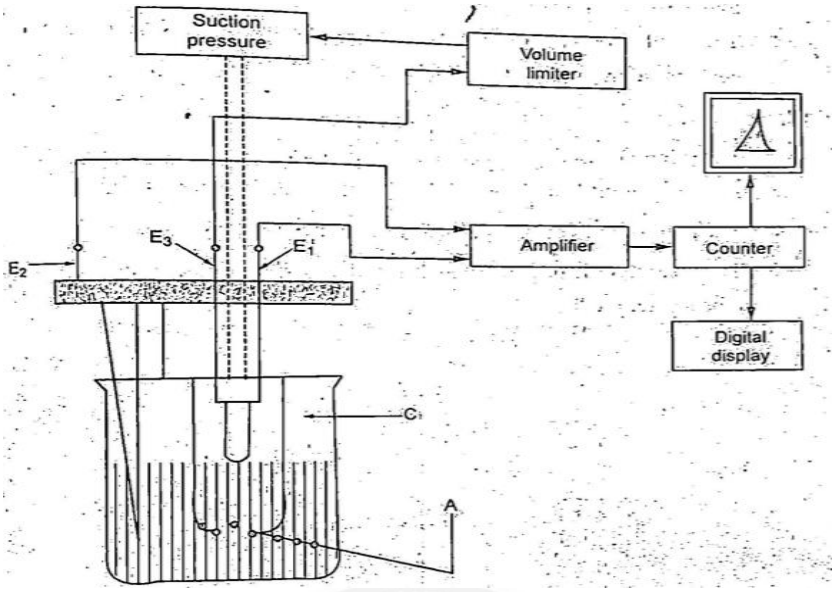

Fig: Block diagram of blood cell coulter counter

In above figure, a glass measuring tube 'c' provided with an aperture 'A' is immersed into the suspension. The pressure difference created between the two sides of the aperture draws the suspension to flow through the aperture.

A constant current is normally passed between the electrodes E_1 and E_2. The electric resistance of the liquid measured between these two electrodes changes rapidly when a particle having electric conductance differing from the conductance of the electrolyte passes through the aperture.

This results in the generation of a voltage pulse, which is amplified in a preamplifier of high gain and low noise level. The output signal of this stage goes to a discriminator, which compares the amplitude of the pulse arriving at its input with the pre-set triggering level. If the input signal exceeds the triggering level, the discriminator gives out a pulse of constant shape and amplitude. These pulses go to a counting circuit for the display of the measured parameter.

iv) Laser based cell counting

This technique is used to determine the number of RBC's, WBC's and platelets. The cell volume of the red blood cells and the haemoglobin concentration can also be obtained by this method.

Principle:

The principle used in this laser-based blood cell counting is “the angle of scattered light is different for different sized blood cells”. The blood is diluted and passed through the capillary tube. The laser light is passed through the glass tube and the blood cells in the tube scatter the light.

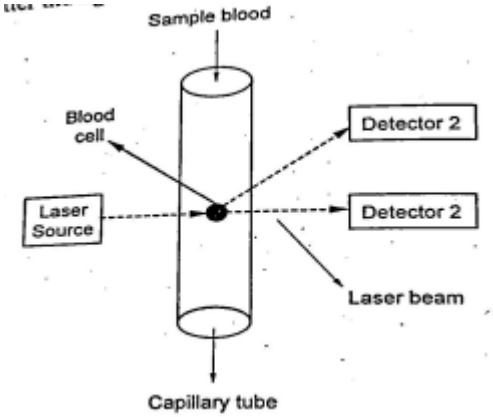

Fig: Laser based cell counting

The scattering angles of platelets and RBC are different. They are detected by two different photo detectors. The detectors are given the digital voltmeter which gives the density of blood cells and platelets. The haemoglobin concentration in the RBC’s also can be measured by this method.