ROHINI
COLLEGE OF ENGINEERING AND TECHNOLOGY
Approved by AICTE and affiliated to Anna University Chennai (An ISO Certified Institution)
Accredited by NAAC with A+ Grade

DEPARTMENT OF BIOMEDICAL ENGINEERING

BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.8 Blood Cell Counter

5.8.1 Types of Blood Cells:

- 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 (cells) to 55 parts of plasma.
- The percentage of cells in the blood is called the haematocrit value or packed cell volume (PCV).
- The majority of the corpuscles in blood are red blood cells (erythrocytes), others being white blood cells (leucocytes) and platelets (thrombocytes).

Erythrocytes (Red Blood Cells):

1. Red blood cells have the form of a bi-concave disc with a mean diameter of about 7.5 μ and thickness of about 1.7 μ. The mean surface area of the cell is about 134 μm^2.
2. There are about 5.5 million of them in every cubic millimetre of blood in men and nearly 5 million in women.
3. In the whole body, there are about 25 billion erythrocytes and they are constantly being destroyed and replaced at a rate of about 9000 million per hour.
4. 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):

1. Leucocytes are spherical cells having a nucleus. There are normally 5000–10,000 white cells per cubic mm of blood but their number varies during the day.
2. They live for seven to fourteen days and there is a rapid turnover, with constant destruction and replacement. Leucocytes form the defence mechanism of the body against infection.
3. They are of two main types: the neutrophils and the lymphocytes.
4. Neutrophils ingest bacteria and lymphocytes are concerned with immunological response.
5. The number and proportion of these types of leucocytes may vary widely in response to various disease conditions.

Thrombocytes (Platelets):

1. Platelets are usually tiny, round, oblong or irregularly shaped cells of the blood with an average diameter of approximately 2 μ.
2. They play an important role in the blood coagulation process.
3. There are usually 250,000–750,000 platelets in every cubic mm of blood.

Calculation of Size of Cells:

1. **Mean Cell Volume (MCV)** is a measure of the average volume of a red blood cell. It is an important parameter in the complete blood count (CBC) and helps in the classification of different types of anemia. MCV is calculated by dividing the total volume of red blood cells by the total number of red blood cells in a given volume of blood.

$$\text{MCV} = \frac{Hematocrit\ (\%)}{Red\ Blood\ Cell\ Count\ (million\ cells/\mu L)}\ X10$$

Normal Range: Adults: 80-100 femtoliters (fL)

2. Mean Cell Haemoglobin (MCH):

Mean Cell Hemoglobin (MCH) is a measure of the average amount of hemoglobin per red blood cell. It is one of the red blood cell indices included in a complete blood count (CBC) and helps in the diagnosis and classification of anemia and other hematological conditions.

$$\text{MCH} = \frac{Hemoglobin\ (g/dL)}{Red\ Blood\ Cell\ Count\ (million\ cells/\mu L)}\ X10$$

Normal Range: Adults: 27-33 picograms (pg) per cell

3. Mean Platelet Volume (MPV):

Mean Platelet Volume (MPV) is a measure of the average size of platelets in the blood. It is included in a complete blood count (CBC) and provides information about platelet production in the bone marrow and platelet activation.

Normal Range: Adults: 7.5-11.5 femtoliters (fL)

METHODS OF CELL COUNTING

- ✓ Microscopic Method
- ✓ Automatic Optical Method
- ✓ Electrical Conductivity Method

Microscopic Method :

1. 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.
2. Commonly known as the counting chamber technique, it suffers from several common drawbacks.
3. Apart from the inherent error of the system, which may be about 10%, there is an additional subjective error of ±10% entailing poor reproducibility of the

results. Furthermore, the lengthy procedure involved results in the rapid tiring of the person making the examination.

4. Another problem with microscopic counting is that the data gathered by this measurement is not directly suitable for storage or for further processing and evaluation.

Procedure:

1. **Preparation of the Blood Sample**

1. **Collect the Blood Sample:** Draw a blood sample and place it in an EDTA tube to prevent clotting.
2. **Mix the Sample:** Gently invert the tube several times to mix the blood with the anticoagulant thoroughly.

2. **Dilution of the Blood Sample**

1. **Prepare Diluting Fluid:** Choose an appropriate diluting fluid based on the type of cells you are counting.
2. **Dilute the Blood:** Using a pipette, draw a small amount of blood and mix it with the diluting fluid. The dilution ratio depends on the type of cells:
    - For RBCs: Typically, a 1:200 dilution.
    - For WBCs: Typically, a 1:20 dilution.

3. **Loading the Hemocytometer**

1. **Clean the Hemocytometer:** Wipe the hemocytometer and cover slip with disinfectant wipes to remove any dust or contaminants.
2. **Charge the Hemocytometer:** Place the cover slip on the hemocytometer. Using a pipette, fill the counting chamber with the diluted blood sample. Allow the sample to spread evenly under the cover slip by capillary action.
3. **Let it Settle:** Allow the cells to settle for a few minutes to ensure an even distribution in the chamber.

4. Counting the Cells

1. **Microscope Setup:** Place the hemocytometer on the microscope stage and focus on the grid lines using low power (10x objective).
2. **Switch to High Power:** Once focused, switch to high power (40x objective) for detailed counting.
3. **Identify the Grid:** Identify the central grid area for RBC counting and the four large corner squares for WBC counting.

5. Counting Procedure

1. **Count Red Blood Cells (RBCs):**
   - Focus on the central large square (divided into 25 smaller squares).
   - Count the cells in five of the smaller squares (four corners and one center).
   - Multiply the counted number by 10,000 to get the number of RBCs per microliter (µL) of blood.
2. **Count White Blood Cells (WBCs):**
   - Focus on the four large corner squares.
   - Count all the cells within these squares.
   - Multiply the counted number by 50 to get the number of WBCs per microliter (µL) of blood.

6. Calculations

1. RBC Count Calculation:

$$\text{RBC count} = \left(\frac{\text{Number of RBCs counted in 5 squares}}{5} \right) \times 10,000$$

2. WBC Count Calculation:

$$\text{WBC count} = \left(\frac{\text{Number of WBCs counted in 4 squares}}{4} \right) \times 50$$

Automatic Optical Method:

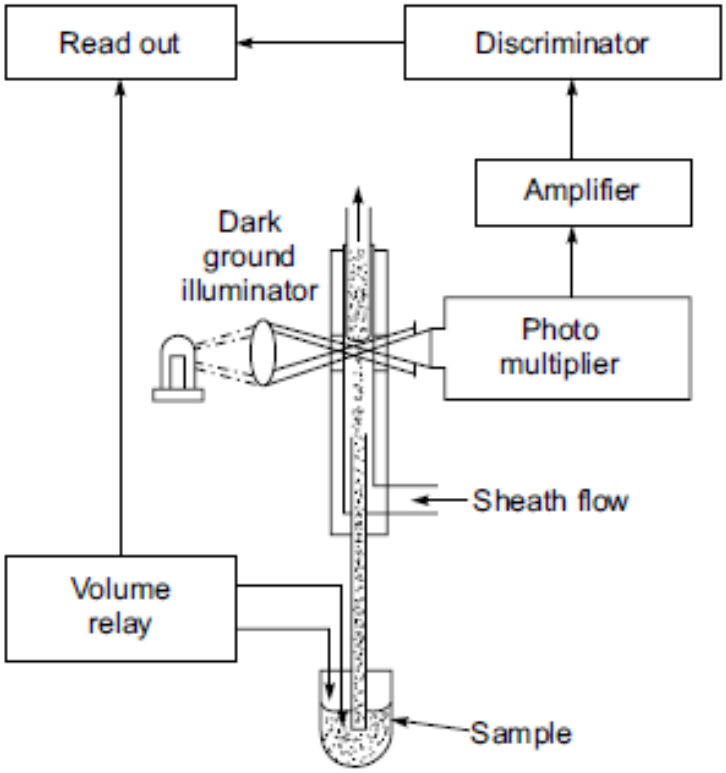

Fig. 5.8.1 Optical method of counting cells

- The method is based on collecting scattered light from the blood cells and converting it into electrical pulses for counting.
- Fig. 5.8.1 shows one type of arrangement for the rapid counting of red and white cells using the optical detection system.
- A sample of dilute blood (1:500 for white cells and 1:50,000 for red cells) 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.
- 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.

- These signals are amplified in a high input impedance amplifier and fed to an adjustable amplitude discriminator.
- The discriminator provides pulses of equal amplitude, which are used to drive a digital display.
- Instruments based on this technique take about 30 s for completing the count
- The instruments require about 1 ml of blood sample.

Electrical Conductivity Method :

- Blood cell counters, operating on the principle of conductivity change, which occurs each time a cell passes through an orifice, are generally known as ***Coulter Counters***. The method was patented by Coulter in 1956.
- The underlying principle of the measurement is that blood is a poor conductor of electricity whereas certain diluents are good conductors. For a cell count, therefore, blood is diluted and the suspension is drawn through a small orifice.
- By means of a constant current source, a direct current is maintained between two electrodes located on either side of the orifice.
- As a blood cell is carried through the orifice, it displaces some of the conductive fluid and increases the electrical resistance between the electrodes.
- A voltage pulse of magnitude proportional to the particle volume is thus produced. The resulting series of pulses are electronically amplified, scaled and displayed on a suitable display.
- A constant current is normally passed between the electrodes E1 and E2. Therefore, 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.

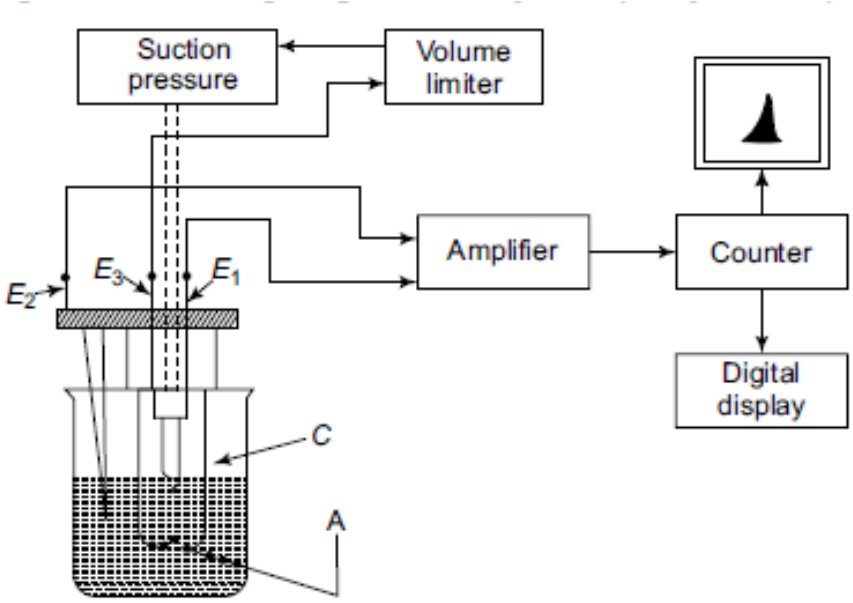

Fig. 5.8.2 Block diagram of blood cell Coulter counter

- 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 preset 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.

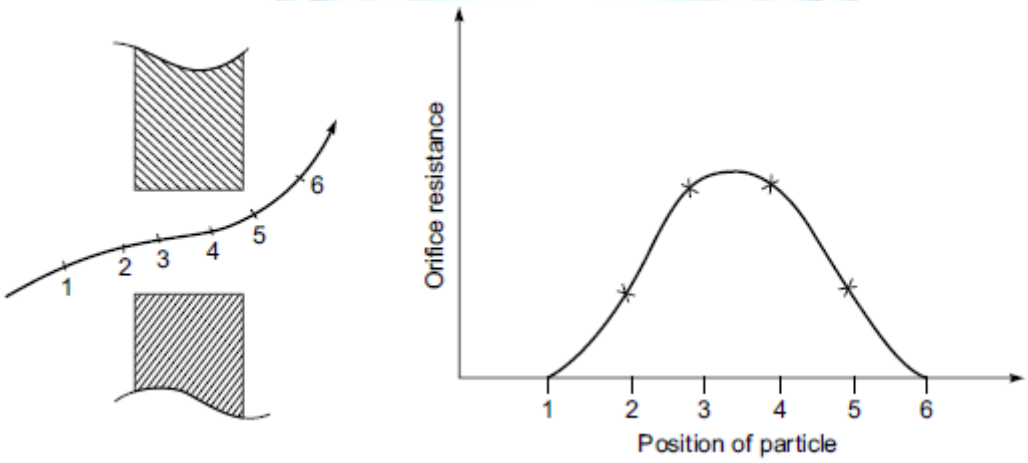

Fig. 5.8.3 The sequence of building up the pulse in terms of increase in resistance at different positions the cell has with respect to the orifice

- Fig. 5.8.3 shows the sequence of building up the pulse in terms of increase in resistance at different positions of the cell with respect to the orifice.
- One great advantage of this instrument is that the clogging of the capillary is greatly eliminated by applying a bi-directional flow during the measurement procedure.
- The number of particles N in a unit volume is determined from the relation,

$$N = \frac{HLE}{V}$$

where

H = factor of dilution
L = scaling factor of the counter
V = measured volume
E = result displayed on the digital display.

- The Coulter counters are usually provided with an LCD to display the pulse information, which has passed through the amplifier, and acts as a visible check on the counting process indicating instantaneously any malfunctions such as a blocked orifice.
- **Errors in Electronic Counters**

  There are a number of errors that may occur in the electronic cell counting technique. Briefly, these errors are categorized as follows:

  - ✓ Aperture Clogging:
  - ✓ Uncertainty of Discriminator Threshold
  - ✓ Coincidence Error
  - ✓ Settling Error
  - ✓ Error in Sample Volume
  - ✓ Error due to Temperature Variation
  - ✓ Biological Factors
  - ✓ Dilution Errors
  - ✓ Error due to External Disturbances

****