**COLLEGE NAME & CODE : Periyar Arts College, Cuddalore-01 & 105**
**COURSE NAME & CODE : II B.Sc., Microbiology & U26**
**SEMESTER : III**
**SUBJECT TITLE & CODE : HEMATOLOGY AND BLOOD BANKING & BSMB 33**

**UNIT-III**

Coagulation Mechanism: Factors: Bleeding time, Clotting time.

Haemotological indices:-

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|---|---|
| Packed cell volume : | Wintrobes / Micro HCT method - Mean corpuscular Volume – Mean corpuscular haemoglobin – Mean Corpuscular haemoglobin concentration - Volume index- volume thickness index - Mean corpuscular diameter -saturation index. |
| Erythrocyte sedimentation: | Principle-Determination: Wintrobes / Westergren Method - advantages / disadvantages – Factors influencing. |

# COAGULATION (or) HEMOSTASIS

(Ref: Bloody easy-Coagulation Simplified, Lesley Black & Rita Selby, Pub: ORBCON. Ontario, 2013)

**Blood Clotting Process (Mechanism)**

Blood flows through the blood vessels to deliver the needed oxygen and nutrients to the different cells in the body. The blood clotting process or coagulation is an important process that prevents excessive bleeding in case the blood vessel becomes injured. It plays a crucial role in repairing blood vessels.

**CLOTTING FACTORS**

- Clotting factors are components found in plasma that are linked to the blood clotting process.
- These factors are named and numbered based on their discovery.
- Though there are a total of 13 numerals, there are only 2 clotting factors.
- Factor VI was discovered to be part of another factor.

The clotting factors are

i. Factor I (fibrinogen),
ii. Factor II (prothrombin),
iii. Factor III (tissue thromboplastin or tissue factor),
iv. Factor IV (ionized calcium),
v. Factor V (labile factor or proaccelerin),
vi. Factor VII (stable factor or proconvertin), and
vii. Factor VIII (antihemophilic factor).

- Hemostasis has three major processes namely the constriction of blood vessels, activity of the platelets, and activity of the proteins found in blood (clotting factors).

**a) Injury**

– The first phase of the blood clotting process is injury or when a blood vessel becomes damaged. This can be in the form of a small tear in the blood vessel wall that may lead to bleeding.

**b) Blood Vessel Constriction**

– The body will constrict the blood vessel to control blood loss. It will limit the blood flow to the affected area.

**c) Platelet Plug**

– In response to the injury, the body activates platelets. At the same time, chemical signals are released from small sacs in the platelets to attract other cells to the area.
– They make a platelet plug by forming a clump together. A protein called the von Willebrand factor (VWF) helps the platelets to stick together.

**d) Fibrin Clot**

– When a blood vessel becomes injured, the coagulation factors or clotting factors in the blood are activated.
– The clotting factor proteins stimulate the production of fibrin, which is a strong and strand-like substance that forms a fibrin clot. For days or weeks, this fibrin clot strengthens and then dissolves when the injured blood vessel walls close and heal.

# BLEEDING TIME & CLOTTING TIME

## BLEEDING TIME

- Bleeding time is defined as the time taken for a standard skin wound to stop bleeding.
- Upon vessel injury, platelets adhere and form a haemostatic platelet plug..
- So the duration of bleeding from standard puncture wound of the skin is a measure of the function of platelets as well as the integrity of the vessel wall.
- There are several methods of performing the bleeding time:
    1. Duke method
    2. IVY method

### 1. DUKE METHOD

- It is the most frequently used method to determine BT in clinical laboratories as it is easy to perform and requires minimal equipment and laboratory skills.

**Requirements:**

i. Equipment for sterile finger puncture
ii. Blotting paper or filter paper
iii. Stopwatch

**Procedure:**

i. Clean the lobe of the ear or tip of a finger with alcohol and let dry.
ii. For ear, glass slide is placed behind the ear lobe and held firmly in place this provide a firm site for incision.
iii. Discard the glass slide if ear lobe has been incised.
iv. Pierce the ear lobe (or tip of a finger) with the lancet, making the incision 3 mm deep
v. Start the stopwatch.

**Normal Values:**

Normal range of BT by the Duke's method varies from 1 to 5 minutes.

### 2. IVY METHOD

- It is more reliable than the Duke method, but it is more painful to the subject.
- Skill is required for using a sphygmomanometer

**Normal Values:**

Normal values of 1 to 6 minutes.

## COAGULATION TIME:

- It is the time required for blood to clot without the presence of any substance.
- Clotting time is usually determined by two methods
    i. Capillary tube method
    ii. Lee-White (venepuncture) method

### 1. Capillary tube method

**Requirements:**

i. Material for Sterile finger prick
ii. Capillary tubing (10-15 cm in length and 1.5 mm in dm) without anticoagulant

**Procedure:**

i. Clean a finger with spirit and allow the spirit to dry.
ii. Pricked the finger by lancet. Remove the first drop of blood.
iii. Squeeze the finger to obtain a larger drop of blood and fill the capillary tube with blood.
iv. The capillary tubes are sealed plasticine and immersed in water bath at 37 ºC.
v. After one minute start breaking small pieces of the capillary tube every 30 second until a fibrin thread is seen between the two broken ends.

**Normal Values:**

By this method, the normal clotting time is 5 to 10 minutes at 37 ºC.

# HEMATOLOGICAL INDICES

**(Red blood cell indices)**

- Red blood cell indices are blood tests that provide information about the hemoglobin content and size of red blood cells.
- Abnormal values indicate the presence of anemia and which type of anemia it is

**Mean corpuscular Volume**
**Mean corpuscular haemoglobin**
**Mean Corpuscular haemoglobin concentration**
**Volume index**
**Volume thickness index**
**Mean corpuscular diameter**
**Saturation index**

## Mean corpuscular volume (MCV)

- Mean corpuscular volume (MCV) is the average volume of a red blood cell and is calculated by dividing the hematocrit (Hct) by the concentration of red blood cell count.
- Normal range: 80-100 fL
- The normal range for MCV is 80–100 fL.

$$MCV = \frac{Hct}{(RBC)}$$

**or**

$$MCV = \frac{PCV \times 10}{\text{RBC count in million/mm}^3}$$

- **Normal range** = 74 - 95 $\mu m^3$
- The mean corpuscular volume is a part of a standard complete blood count.
- In patients with anemia, it is the MCV measurement that allows classification as either a microcytic anemia (MCV below normal range), normocytic anemia (MCV within normal range) or macrocytic anemia (MCV above normal range).
- For further specification, it can be used to calculate **red blood cell distribution width (RDW).** The RDW is a statistical calculation made by automated analyzers that reflects the variability in size and shape of the RBCs.

## Mean corpuscular hemoglobin concentration

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin per unit volume of red blood cells and is calculated by dividing the hemoglobin by the hematocrit.

$$MCHC = \frac{Hb}{Hct}$$

- Normal range: 32-36 g/dL

**Mean corpuscular haemoglobin (MCH)**

- Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin (Hb) per red blood cell and is calculated by dividing the hemoglobin by the red blood cell count.
- Normal range: 27-31 pg/cell
- It is calculated by dividing the total mass of hemoglobin by the number of red blood cells in a volume of blood.

$$MCH = \frac{(Hb \text{ in } g\% \times 10)}{\text{RBC count in million/mm}^3}$$

- A **normal MCH value** in humans is 27 to 31 picograms/cell.
- The amount of hemoglobin per RBC depends on hemoglobin synthesis and the size of the RBC.
- The MCH decreases when Hb synthesis is reduced, or when RBCs are smaller than normal, such as in cases of iron-deficiency anemia.

(Ref: https://books.google.co.in/books?id=gH_rS8tuz8wC&pg=PA59&lpg=PA59&dq=Volume+index+Volume+thickness+index+Mean+corpuscular+diameter+saturation+index&source=bl&ots=sO_h-foHXR&sig=ACfU3U2EmD9rEfviAb9REgoC7H0o3E-dzQ&hl=en&sa=X&ved=2ahUKEwj5yc6a2OLsAhUhwjgGHS6qDrE4ChDoATAFegQIARAC#v=onepage&q&f=true)

# Erythrocyte sedimentation rate (ESR)

- The ESR is a simple non-specific screening test that indirectly measures the presence of inflammation in the body.
- It reflects the tendency of red blood cells to settle more rapidly in the face of some disease states, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins.

**METHOD**

When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the RBCs – under the influence of gravity - settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).

There are two main methods used to measure the ESR:

a. the Westergren method and
b. the Wintrobe Method.

Each method produces slightly different results. Most laboratories use the Westergren method.

**a. WESTERGREN METHOD:**

- The Westergren method requires collecting 2 ml of venous blood into a tube containing 0.5 ml of sodium citrate.
- It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C.
- The blood is drawn into a Westergren-Katz tube to the 200 mm mark.

**b. Wintrobe method:**

- The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long.
- EDTA anticoagulated blood without extra diluent is drawn into the tube, and the rate of fall of red blood cells is measured in millimeters after 1 hour.

**Average values**

- in healthy men are: <15mm/hr
- in healthy females: <20mm
- The values are slightly higher in old age, in both genders. **DISADVANTAGE:**

Note that the ESR denotes merely the presence of tissue damage or disease, but not its severity; it may be used to follow the progress of the diseased state, or monitor the effectiveness of treatment.

**Some interferences which increase ESR:**

- increased level of fibrinogen, gamma globulins.
- technical factors: tilted ESR tube, high room temperature.
- Chronic inflammatory disease (collagen and vascular diseases) increases ESR.

**Some interferences which decrease ESR:**

- abnormally shaped RBC (sickle cells, spherocytosis).
- technical factors: short ESR tubes, low room temperature, delay in test performance (>2 hours), clotted blood sample, excess anticoagulant, bubbles in tube.
- Polycythemia decreases ESR.