Hae.

Lesson → 1

# Erythrocyte Sedimentation Rate (ESR) and Packed cell volume (PCV).

1) **Introduction :-** Blood is a combination of plasma and cells that circulate through the entire body. Plasma contain 90% water and 10% proteins, carbohydrates Lipids, enzymes, vitamins.

- Red Blood cell (erythrocytes)
- White Blood cell (leucocytes).
- Platelets (thrombocytes)

2) **Erythrocytes Sedimentation Rate (ESR) :-**
The rate at which red Blood cell Settle in an anticoagulated undisturbed Blood sample under standard conditions.

- Increase in plasma fibrinogen.
- Changes in the positive charges of plasma.
- Changes in erythrocyte surface, electric charges. Shape etc.

⇒ **Stage of erythrocyte Sedimentation :->**

**Stage – 1 :-** Rouleaux formation :- It occurs during first ten minutes.

**Stage ÷ 2 :-> Sedimentation or Settling :->** It Occurs during next 40 minutes after Stage ÷ I

Stage – III :- Stage of packing :-> It occurs during next 10 minutes after stage – II Sedimentation slows and cell start to pack at the bottom of the tube.

3) Factors which increase ESR :-
- Increased level of fibrinogen, gamma globulins.
- Technical factors such as tilted ESR tube High room temperature.

4) Factors which decrease ESR :-
- Abnormal shaped RBC's eg Sickle cell, Spherocytosis.
- Technical factors such as short ESR tube low room temperature, clotted blood sample excess anticoagulant, bubbles in tube etc.

:- Methods of ESR Estimation :-

1) Wintrobe's Methods :- This method is less sensitive as compared to the Winter gren method but it has the advantage that it provides two results ie. ESR and PCV simultaneously.

⇒ Equipments :- • Wintrobe's tube.
> Length — 110 mm.
> Internal diameter — 2.5 mm.
> Graduated in mm upto 100 mm.
> Mark '0' mm towards the top side and 100mm towards the bottom side.
• Wintrobe's tube Stand.
• Pasteur pipette with long neck.
• Timer.

⇒ Specimen :- 5 mL. of venous Blood after overnight fast, mixed with EDTA / double oxalate anticoagulant.

Procedure :- i) Mix the anticoagulant Blood throughly.
ii) Place the tube vertically in Wintrobe's tube Stand.
iii) Take reading for 1 Hr at an interval of 15 min.
iv) At the end of one Hour, note the level of red cells from '0' mark at the top.

⇒ Normal Range :- Male :- 0 to 9 mm
female :- 0 to 20 mm

⇒ Precautions :- • There should be no air bubble in the Blood.
• Clean the Wintrobe's tube and Pasteur pipette immediately after use and dry at 37°c.

⇒ **Merit :-** • It provides two result simultaneously ie ESR and PCV.

⇒ **Demerits :-** • Less sensitive than westergren methods.
• More practical for demonstration purposes.

2) **Westergren Method :-** This method was developed by the scientist westergen in 1921. This method for measuring ESR. has also been recommended by the International council of standardisation (ICSH) in Haematology. Most Laboratories use this method because it is a very sensitive method.

⇒ **Regent :-** Sodium citrate 3.8 % w/v.

⇒ **Requirements :-** • westergen's tube.
• westergen's tube stand.
• Suction device
• Timer.

⇒ **Procedure :-** • Mix the anticoagulated Blood thoroughly.
• Draw the Blood into the tube upto 0 mark with the help of Rubber bulb.
• Wipe out Blood from bottom of the tube with cotton.
• Leave the tube undisturbed for 1 Hour.
• Take reading at an interval of 15 mint.

. At the end of 1 Hour, Note the level of red Blood cell from 0 mark at the top.

⇒ Normal range — Male — 0–15 mm
Female — 0–20 mm.

⇒ Precautions — . Clean the westergen's tube / pipette immediately after use as per guidelines and dry at 37°c.

. westergen's tube must be in vertical position during the test.

⇒ Merits — . More accurate results.

⇒ Demerits — . Mouth pipetting is dangerous.
. PCV test cannot be conducted Simultaneously

3) Landau's microsedimentation method.

## Factors Affecting ESR.

1) Physiological factors.

. Plasma factors.
. Red cell factor
. Rouleaux formation.
. Age
. Sex
. Pregnancy
. obesity
. Smoking.

⇒ Plasma factors :- i) Plasma albumin retard ESR
ii) Plasma cholesterol accelerates ESR.

⇒ Red cell factors :- i) Abnormally shaped RBCs (Sickle cell) decrease ESR.
ii) Anaemia :- Severe anaemia accelerates ESR.

⇒ Rouleaux formation :- It increases ESR.
- Age :- ESR steadily increases with age.
- Sex :- ESR is higher in females than males.
- Pregnancy :- ESR increases during pregnancy.
- obesity :- ESR is higher in obesity.
- Smoking :- ESR is higher in smokers.

2) Laboratory factor :-
- Temperature
- Time
- Anticogulants
- Tube factor.
- Tilting of tube
- Vibration
- Sunlight.

⇒ Tilting of tube :- It leads to increased ESR.

⇒ Vibration :- Vibration during the determination of ESR reduce the ESR.

⇒ Sunlight :- Direct sunglight increases ESR.

⇒ Tube factor :- ESR is greater with longer tube.
ESR is less with short tube.

⇒ Time :- Delary in ESR test for more than two Hours after Blood collection retards ESR.

## Packed cell Volume (PCV)

⇒ **Packed cell Volume (PCV) :-** It is defined as the ratio of volume of red Blood cell to the volume of whole Blood. It is expressed as percentage.

⇒ **Normal values :-** Adult male :- 42 – 54 %
Adult female :- 38 – 46 %.

## Methods of Estimate PCV

1) **Microhaematocrit Method :-** This method involves using small quantity of whole Blood, Capillary tube and High speed centrifuge for the determination of PCV. It is beneficial for patients from whom Blood Collection is difficult.

⇒ **Principle :-** When EDTA treated or Heparinised Blood is centrifuged in a capillary tube at 10,000 R.P.M. for five mint, the Blood separat in capillary tube into three layers, top – Plasma layer, Buffy coat (middle) and RBC is at the Bottom.

⇒ **Requirements** :- • Haematocrit capillary tube.
• Microhamatocrite centrifuge.
• Haematocrite reader.
• Modelling clay (Plasticine)

⇒ **Procedure** :- • Fill two capillary tube approximately 2/3 to 3/4 full with the well mixed Blood sample.
• Seal dry end of the capillary tube with modelling clay.
• Duplicate tube must be placed opposite each other for balane.
• Centrifuge at 10,000 to 15,000 RPM for 5 min.

⇒ **Normal values** :- Adult male 42-54 %
Adult female 38-46 %

⇒ **Precautions** :- i) Centrifugation speed must be constant
ii) Blood and anticoglulant must be mixed thoroughly

**Brief of Different Layer of Blood** :-

1) **Plasma layer (top)** :- It is the top layer. Normal plasma is straw coloured. If it is :-
• Colourless :- Iron deficiency.
• Pink :- Haemolysis.
• Yellow :- Jaundice
• Milky :- Hypertriglyceridemia.

ii) Buffy Coat :- . upper coat :- It consists of platelets.
. Lower layer :- WBC . It is generally 1 mm thick.

iii) PCV :- It is defined as the ratio of volume of Red Blood cell to the volume of whole Blood. It is expressed as percantage.

⇒ Equipment :- . Wintrobe tube.
. Pasteur pipette or syringe with Needle.
. Timer.

⇒ Procedure :- . Mix the anticoagulated Blood throughly.
. Centrifuge at 3000 RPM for 30 min.
. Take the reading of the length of column of the Red cells.
. Haematocrit can be expressed either ar percentage or as a fraction of the total volume of Blood sample.

⇒ Precautions :- . Centrifugation speed must be Constant.
. Air bubbles must be avoided while filling the wintrobe tube with sample of Blood.
. wash the wintrobe tube and Pasteur pipette immediately after use as per the guide lines and dry at 37°c.

⇒ Sources of Error :- • Anticoagulant and Blood Sample not mixed thoroughly.
• High concentration of anticoagulant.
• failing to note the correct reading on Haem atocrite tube.

Lesson → 7.

# Red cell Indices MVC, MCH, MCHC.

1] **Red Blood indices** :- Red Blood indices are the measure of size and Haemoglobin content of the red Blood cell.

2] **Mean cellular volume (MCV)** :- It is defined as the measure of average size of Red Blood cell. It is express as the volume in cubic microns or femtolitres (fl) of an average red Blood cell. 1fl = $10^{-15}$ L.

**Normal range :** 80-100 cubic microns

**(MCV) :-**

$$\frac{PCV\% \times 10}{RBC's \text{ (Millions per cu mm)}}.$$

3] **Mean corpuscular (cellular) Haemoglobin (MCH)** :- It refers to the average quantity of Haemoglobin present in the single red Blood cell. It is expressed in picograms (pg.) 1pg = $10^{-12}$ g.

**Normal Range :** 27-31 pg. per cell.

**MCH :-**

$$\frac{\text{Haemoglobin} \times 10}{\text{RBC's (Millins per cu mm)}}.$$

4] **Mean Corpuscular Haemoglobin (MCH)** :- It describes the average concentration of Haemoglobin in give volume of Red Blood cell. It is expressed as percentage of packed cells (g/dL).

MCHC = $\frac{\text{Haemoglobin (g/dL)} \times 100}{\text{PCV \%}}$

Normal range :- 32–36 %

## Red cell Distribution WIDTH (RDW)

RDW :- This test is the measurement of the Range in the volume and size of RBC.

⇒ Normal Range :- Adult male — 12.2 to 16.1 %
Adult female — 11.8 to 14.5 %

| S. No. | Size / Haemoglobin content of RBCs | Anamia |
|---|---|---|
| 1 | Smaller size than the Normal Range. | Microcytic |
| 2 | Large size than the Normal Range | Macrocytic |
| 3 | Normal Size. | Normacytic |
| 4 | Less Haemoglobin than the Normal Range. | Hypochromic |
| 5 | High Haemoglobin than the Normal Range | Hyperchromic |
| 6. | Normal Range of Haemoglobin. | Normochromic |

## Wintrobe Determination of Red cell Indices

## Interpretation :-

- The RBC indices measure the size, shape and physical characteristics of the RBC's. RBC indices help to diagnose the cause of anaemia.

- RBC indices are of three types and their abnormal volume indicate the disorders as given below:-

i) MCV :- It is the average of Red Blood cell size. Normal range is 80-100 femtolitres. The MCV may be Normal, low or high.

ii) MCH :- It is the amount of Haemoglobin per red Blood cell. Normal range is 27-31 picogram per cell.

iii) MCHC :- It describes the average concentration of Haemoglobin in a give volume of Red Blood cell. Normal range is 32-36%.

Lesson → 3.

# Supravital Stain and Reticulocytes Counting.

**1) Introduction :-** Reticulocytes are immature RBC. In the process of erythropoiesis (Red cell formation), reticulocytes develop and mature in the bone marrow and then circulate for about a day in the Blood Stream before developing into the Mature RBC.

## 2) Supravital Staining

**Supravital Staining :-** This method of staining is used in microscopy to examine living cell that have been removed from an organism.

## 3) Staining Solutions for Supravital Staining :-

**i) New methylene blue :-**

| | |
|---|---|
| New methylene blue — | 1g |
| Sodium citrate 3% w/v — | 20 mL |
| Sodium chloride 0.9 % w/v — | 80 mL |

Mix all ingredients to make the solution ÷ filter before use.

- New methylene Blue. : Staining agent
- Sodium citrate : Prevents coagulation
- Sodium chloride : Provides isotonicity.

**ii) Brilliant cresyl blue :-**

Brilliant cresyl blue : 1g.

Sodium citrate 3.8% w/v : 20 mL
Sodium chloride 0.9% w/v : 80 mL

Mix all ingredients to make the solution. filter before use.
- Brilliant cresyl blue. : Staining agent
- Sodium citrate. : Prevents coagulation.
- Sodium chloride. : Provides isotonicity.

**4] Interpretation :-** Reticulocytes are immature RBC. In the process of erythropoiesis, and mature in the bone marrow an then circulate for about a day in Blood stream before developing into the mature red Blood cell.

**Normal Range :-** Adult : 0.2 – 2 %.
Infants : 2 – 6 %

⇒ **Reticulocytosis :-** It indicates increased count of reticulocytes.

⇒ **Reticulocytopenia :-** It indicates decreased count of reticulocytes.

## :- Variations in physiological Value :-

**1] Haemoglobin :-** It is the protein molecule of Red Blood cell. that carries oxygen from the lungs to the body tissue and return carbiex. carbon dioxide from the tissue back to the lungs.

⇒ **Normal Range** :- Adult males : 14 to 18 gm·/dl
Adult women : 12 to 16 gm /dl
New born : 17 to 22 gm·/dl

⇒ **(i) Lower than normal Haemoglobin level** :- A lower physiological level than normal is referred to as anaemia or low red blood count.

**(ii) Higher than normal Haemoglobin level** :-
The Higher level of Haemoglobin in Blood is due to hypoxemia. ie - low level of oxygen in the Blood. Medical Condition that can cause high Haemoglobin level.

**2) Packed cell volume (PCV)** :- ⇒ It is defined as the volume of red cell in the given Sample of Blood. It is expressed as percentage.

**Normal Range** :- Adult male : 42 - 52%
Adult female : 36 - 46 %

**3) Physiological variation in TLC / WBC's** :-
Leucocytes are also known as WBC. These are immune cells that circulate in the Blood and lymphatic system. They protect the Body from bacteria, parasitic and fungal infections.

i) Leucopenia :- It indicates the decreased count of leucocytes.

ii) Leucocytosis :- It indicates the increased count of Leucocytes.

## Variations in platelets count (Thrombocytes count) :-

1) Thrombocytopenia :- It indicates the discreased count of platelets.

2) Thrombocytosis :- It Indicates the increased count of platelets.

## Reticulocytes characteristics :-

- Comprise about 1% of the total RBC's.
- They are about 8% larger than the red cell into which they mature.
- Develop and mature in the bone marrow.
- Do not possess nucleus.
- Metabolic activity present.
- move at slower speed as compared RBC's.

Jyotika
4/12/22

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# Leson → 4.

## Anaemias.

1) **Anaemia** :- Anaemia is a condition in which the number of RBC is or amount of Haemoglobin in the Blood fall the Normal levels.

⇒ **The normal count of RBC is** :-

| | | |
|---|---|---|
| Adult male | : | 4.5 — 5.9 million/cu mm |
| Adult female | : | 4.1 — 5.1 million/cu mm |
| At birth | : | 4.0 — 5.6 million/cu mm |

⇒ **The normal range of Haemoglobin** :-

| | | |
|---|---|---|
| Male | : | 13.5 — 17.5 |
| Female | : | 11.5 — 15.5 |
| Infant | : | 9.5 — 12.5 |
| children | : | 11.0 — 13.5 |
| Male (old age) | : | 11.5 — 14.0 |
| Female (old age | : | 10.5 — 13.0. |

- Haemoglobin levels change with age and sex.

⇒ The red Blood cell indices help to determine the type of Anemia.

⇒ **3 types Anaemia and Normal value.**

| | | |
|---|---|---|
| MCV | — | 80 — 100 cubic microns |
| MCH | — | 27 — 31 pg. per cell |
| MCHC | — | 32 — 36 %. |

⇒ **Symptoms of Anaemia** :- . fatigue.
. Shortness of breath

. Weakness.
. Pale or yellowish skin.
. Headache
. Cold hands and feet.

⇒ Signs of Anaemia :-
. Dark urine
. Pallor.
. Glossitis.
. Koilonychia.

⇒ More serious sign of symptoms :-
. fever.
. Heart problem.
. Enlarged liver and spleen.
. Jaundice.

## Anaemia types and Causes :-

There are several different types of anaemia Each type cause the number of RBC in circulation to drop. As such sign and systoms of each type are similar.

. Anaemia caused by Blood loss.
. Anaemia caused by decreased or faulty red Blood cell production.
. Anaemia caused by destrution of RBC.

## Classification of Anaemia :-

1) Aetiology :- It a based on the decreased level of Haemoglobin. The causes responsible for decreased level of Haemoglobin.

1) Blood loss 2) Decreased RBC production
3) Increased RBC destruction.

⇒ **Morphological classification** :- It is based on the size of RBC. eg (MCV). on this basis, anaemia is divided into three group.

- Microcytic (small RBC's)
- Normocytic (Normal size RBC's)
- Macrocytic (Large RBC's).

⇒ **Aetiological classification** :-

i) **Haemorrhagic anaemia** :- Blood loss is common causes of anaemia. It may be due to ; vomiting of Blood, cancer etc.

- Acute Haemorrhagic anaemia eg. Physical injury surgery etc.
- Chronic Haemorrhagic anaemia :- It due to Blood loss for longer period.

2) **Dyshaemorrhagic anaemia** :- This type of anamia is due to decreased RBC's production. It is so named because there is no loss of Blood.

'i) Insufficient production of RBC's.
Ineffective production of RBC's
Bone marrow disease anemia.

(4) Classification of Anaemias

- on the basis of cause
  - Blood loss
    eg: Physical injury surgery
  - Decreased RBC's Production
    eg: Bone marrow infiltration. G.P. Nutrients.
  - Increased RBC's destruction
    eg: Sickle cell disease, Haemoglobin. etc.
- on the basic of Morphology
  - Microcytic Anemia MCV (<80)
  - Normocytic Anaemia MCV (80-100)
  - Macrocytic Anaemia MCV (>100)

Haemorrhagic Anaemia

Pyshaemorrhagic Anaemia

Haemolytic Anaemia

5) Haemolytic Anaemia — It refers to the destruction of red blood cell due to the reduction in the life span of RBC's ie - below 100 days (normal 100 - 120 days).

When RBC's production in the Bone marrow does not keep pace with the level of Haemolysis, then haemolytic Anaemia occurs.

i) Inherited Haemolytic anaemia — It pertain to the factor present in side RBC's. It is further classified based on the site of inherited.

(A) Morphological abnormalities e.g membrane abnormalities.

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(B) Metabolic deficience e.g enzymatic defects. deficiency-

(C) Haemoglobin abnormalities e.g Sickle cell disease

ii) Acquired Haemolytic anemias :- It pertains to the factors outside the RBCs.

(A) Immune Haemolytic anemias :- eg. warm and cold Non-immune Haemolytic anemias.

(B) Non-immune Haemolytic anemias :- eg. mechanical trauma, infections and drugs etc.

6) Morphological classification :-

i) value of red Blood indices such as :-

- Mean corpuscular value (MCV) :- The normal range is 80-100 fl.
- Mean cellular Haemoglobin (MCH) :- The normal range is 27-31 pg per cell.
- Mean cellular Haemoglobin Concemration (MCHC) :- The normal range is 32-36%
- Size of RBCs :- The normal range is 4-6 million (mm3)
- Count of RBCs :- The average size is 7 microns.
- Appearance (Colour) of RBC's :-

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The deviations of values / ranges of the above mantioned parameters indicate.

1) Microcytic anaemia.
2) Normocytic anaemia
3) Macrocytic anaemia.

1) Microcytic anaemia — The anaemia is described as microcytic when MCV is less than 80 fl and size of RBCs is less than 6 microns. Haemoglobin concentration (MCHC) which leads to the appearance of pale RBCs.

Causes —
- Anaemia of chronic disease.
- other Haemoglobinopthies.
- Lead poisoning.
- Sideroblastic anaemia.

1) Laboratory finding: Aplastic anemia — It is a condition when body stop producing enough new Blood cell. ie - RBC, WBC and platelets.

- RBCs WBC's and platelets count low -
- Haemoglobin and/or Haematocrite may be low.
- RBC indices are usually normal.
- Reticulocyte count is low.
- Erythropoietin usally increased.
- Bone marrow show Hypoplasia.
- No Abnormal cells in peripheral Blood smear.

2) Iron deficiency anaemia — The anaemia resulting from iron deficiency is known as microcytic anaemia.

- Low Haemoglobin
- Low Haematocrit
- Low MCV, MCH, and MCHC.
- Low RBC count.
- RBC small, oval shaped with centres.
- Low ferritin.
- Low serum iron.
- High transferrin or total iron-binding capacity (TIBC).

Jyotika
6/12/22

# Lesson → 5.
## Red cell fragility Test.

1) **Red cell fragility** → It refers to the normal tendency of erythrocytes (RBC) to haemolyse under stress.

2) **Haemolysis** → The repture of erthrocytes.

3) **osmosis** → osmosis is a process by which the molecules of solvent pass from a solution of low concentration to a solution of High concentration.

4) **Normal Saline Solution** → It contains 0.9 % Sodium chloride in water. It is isotonic to Blood and tears.

5) **osmotic pressure** → It is defined as the pressure required to stop the water from diffusing through semipermeable membrane by osmosis.

6) **Isotonic Solution** → A solution is said to be isotonic when its effective Solute Concentration is the same as that of another Solution across the Semipermeable membrane.

7) **Hypotonic Solution** → A Solution is said to be Hypertonic Solution if its effectiv

Solute concentration is more than another solution across the semipermeable membrane.

8) Hypertonic Solution → A Solution is said to be Hypertonic Solution if its effective solute concentration is more than another solution across the semipermeable membrane

## → Osmotic fragility Test →

Osmotic → It is (OFT) is used to measure erythrocytes resistance to Haemolysis when exposed to different levels of dilution of a saline solution.

Principle → when erythrocytes are exposed to a hypotonic enviroment, water enters the cell and causes swelling and ultimately lysis.

- The degree of Hgemolysis is measured by determining the absorbance of the Supernatant using spectrophoto meter.

⇒ Materials → whole Blood.
- Collection medium: Sodium heparin tube or lithium heparin tub EDTA
- Methodology: Spectrophotometry / Colorimetry.

⇒ Procedure → . Take 12 Sodium heparin tube and label from 1 to 12.
- Prepare the dilution of stock Solution of

Sodium chloride e.g. 0.9, 0.75, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.2, 0.1 % w/v.

- Put 0.05 ml of Blood in all tubes from 1 to 12
- Mix the contents gently in each tube by inverting several times.
- Allow to stand in incubator at 18–20°C for 30 min.
- Remix and centrifuge.
- A Blood sample for a healthy person is carried out in parallel as a control.
- measure absorption by using spectrophoto meter or colorimeter at 540nm green filters.

% age haemolysis = $\frac{\text{Test absorbance}}{\text{Absorbance of control tube}} \times 100$

⇒ **Interpretation.**

- **Conditions associated with increased osmotic fragility include –**
  - > Hereditary spherocytosis
  - > Autoimmune spherocytosis
  - > Poisoning
  - > Severe burns.
- **Conditions associated with decreased osmotic fragility include –**
  - > Thalassemias
  - > Iron deficiency anemia.
  - > Sickle cell Aneamia.

→ Factors affecting osmotic fragility :-

(i) Intrinsic factors :- i) Age, Sex, breads, location etc.
- Erythrocytes shape, size, number and surface area to volume ration.

ii) Erythrocytes membrane composition, ion transport osmosis etc.

iii) Extrinsic factors include :-
- Type of anticoagulant.
- Storage time and Blood.
- Ambient temperature.
- Xenobiotics.
- Incubation media :- Type, ionic, strength and pH etc.

## Red cell fragility test

It refers to the natural tendency of RBC to Haemolyse under stress.

⇒ Erythrocytes fragility test method :-

1) Mechanical test for erythrocyte fragility :-
The ways by which mechanical stress is applied on erythrocytes, determines the erythrocyte fragility.

- Vigorous shaking of Blood sample with or without glass / metallic bead.

forced passage of Blood sample through narrow aperture.
Subjecting the Blood sample to ultrasonic vibrations.

2) Osmotic erythrocyte fragility test. 6/0

Osmosis 6/0 osmosis is a process by which the molecules of solvent pass from a solution of low concentration to a solution of High concentration.

Jyotika
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