The erythrocyte sedimentation rate (ESR) measures the rate of settling (sedimentation) of erythrocytes in anticoagulated whole blood. Anticoagulated blood is allowed to stand in a glass tube for 1 hour and the length of column of plasma above the red cells is measured in millimeters

## STAGES OF ERYTHROCYTE SEDIMENTATION RATE

There are three stages of erythrocyte sedimentation:

*Stage 1*: Formation of rouleaux or lag phase (10 minutes): Red cells stack together like a pack of coins.

The ESR depends mainly on this stage.

*Stage 2*: Sinking of rouleaux or decantation phase (40 minutes): Rapid and constant sedimentation. The longer the tube, the longer is this stage and higher the value of ESR.

*Stage 3*: Packing of rouleaux (10 minutes): Slow sedimentation.

## FACTORS AFFECTING ERYTHROCYTE SEDIMENTATION RATE

Red cells, composition of plasma, and technical factors affect ESR.

**1. Red cells:** Alteration of ratio of red cells to plasma affects ESR.

Decreased red cell mass in anemia increases ESR.

Conversely, increased red cell mass in polycythemia decreases ESR.

Macrocytes tend to sediment rapidly than microcytes.

Sickle cells and spherocytes are unable to form rouleaux and therefore ESR is low in sickle cell disease

and hereditary spherocytosis. In these conditions ESR is not reliable as an indicator of illness.

**2. Plasma:** The most important factor affecting ESR is the composition of plasma. Increase in fibrinogen, other acute phase proteins (C-reactive protein, haptoglobin, ceruloplasmin, antitrypsin, etc.) and immunoglobulins increase ESR.

Increased proteins in plasma reduce negative charge on the surface of red cells and reduce the zeta potential (the electrical repulsion between red cells); this brings red cells closer together and facilitates rouleaux formation.

Removal of fibrinogen by defibrination and increase of albumin decrease ESR.

**3. Technical factors:** ESR increases with room temperature. Tilting of the tube, and length and bore of the tube affect ESR. Surface vibration, dilution of blood and anticoagulant, mixing of blood also affect the rate of ESR.

**some other factors increasing ESR**

– Old age
– Pregnancy
– Anemia
– Elevated fibrinogen
– Macrocytosis
– Technical factors: High temperature, tilting of ESR tube

**• Factors decreasing ESR**

– Microcytosis

– Low fibrinogen
– Polycythemia
– Marked leukocytosis
– Technical factors: Vibration of tube during test

## CLINICAL SIGNIFICANCE OF ERYTHROCYTE SEDIMENTATION RATE

ESR is elevated in a wide range of organic diseases. ESR is not a specific and diagnostic test for any disease. However, it is helpful in differentiating functional from organic disease. Raised ESR signifies presence of some disease, which needs evaluation. Most of the inflammatory and neoplastic diseases are associated with an increase in ESR. ESR correlates with disease activity and therefore it is helpful in monitoring disease activity and response to therapy in acute rheumatic fever, bacterial endocarditis, tuberculosis, rheumatoid arthritis, temporal arteritis, polymyalgia rheumatica, and Hodgkin's disease. ESR is not significantly raised in typhoid fever, malaria, infectious mononucleosis, angina pectoris, osteoarthritis, acute appendicitis, peptic ulcer, acute allergy, and unruptured ectopic pregnancy. ESR is decreased in polycythemia, congestive cardiac failure, dehydration, sickle cell anemia, hereditary spherocytosis, and hypo fibrinogenemia.

Causes of increased erythrocyte sedimentation rate
1. Infections • Acute rheumatic fever
• Osteomyelitis
• Bacterial endocarditis
• Pyogenic arthritis
• Pelvic inflammatory disease
• Tuberculosis
• Acute hepatitis
2. Inflammatory diseases • Rheumatoid arthritis
• Systemic lupus erythematosus
• Temporal arteritis
• Polymyalgia rheumatica
3. Acute myocardial infarction
4. Malignancy
5. Paraproteinemias • Multiple myeloma
• Waldenström's macroglobulinemia
• Cryoglobulinemia
6. Technical problems • Increased temperature
• Tilted ESR tube

7. Others • Ruptured ectopic pregnancy
• Anemia
• Renal disease with
azotemia
• Administration of dextran or
oral contraceptives

## METHODS FOR ESTIMATION OF ERYTHROCYTE SEDIMENTATION RATE

These include:
• Westergren method
• Wintrobe method

### Westergren Method

*Equipment and Reagent*
1. *Westergren ESR tube*: This is a straight glass pipette measuring 300 mm in length and calibrated in mm from 0-200 (top to bottom). The markings are only over the lower 2/3rds of the tube. The tube is open ended. Internal diameter should not be less than 2.55 mm . The tube should be clean and dry.
2. *Westergren stand*: This holds the tube in a motionless, vertical position.
3. *Anticoagulant-diluent solution*: Trisodium citrate dihydrate is the anticoagulant of choice.
Blood sample should be diluted with trisodium citrate just before the test (1 volume of trisodium citrate to 4 volumes of blood).
*Specimen*: Venous blood is collected in trisodium citrate solution in 4:1 (blood: citrate) proportion. If specimen is kept at room temperature, test should be carried out within 4 hours of blood collection; if stored at 4°C, a delay of up to 6 hours is permissible. Blood anticoagulated with EDTA can be tested within 24 hours if stored at 4°C (provided it is diluted with trisodium citrate before testing).
*Method*
1. Mix anticoagulated blood sample thoroughly. The Westergren tube is filled with the blood sample up to the “0” mark. A rubber bulb or a mechanical device should be used for filling. There should be no air bubbles in the blood.
2. The tube is placed in a strictly vertical position in the ESR stand and left undisturbed for 1 hour. It should not be kept in direct sunlight and should not be subjected to vibrations.
3. After exactly 1 hour, read the height of the column of plasma above the red cell column in mm.
4. Express the result as:
Erythrocyte sedimentation rate = ——— mm in 1 hour.
***Precautions***

1. Use the correct proportion of blood and anticoagulant. Mix blood and anticoagulant thoroughly. There should be no clots and air bubbles in blood.
2. The reference range relates to test performed at room temperature of 18-25°C. If temperature is higher, ESR will increase and different reference range will have to be derived.
3. ESR tube must be kept vertically. Even a slight tilting will increase the ESR.

## REFERENCE RANGES

### Erythrocyte Sedimentation Rate by westergren Method

- Males < 50 years: 0-15 mm in 1 hr
- Females<50 years: 0-20 mm in 1 hr
- Children: 0-10 mm in 1 hr
- Elderly (>50 years): Males: 0-20 mm in 1 hr and females: 0-30 mm in 1 hr.

### Other Methods

*Wintrobe Method*

Wintrobe tube can be used for estimation of both ESR and packed cell volume (PCV). Fill the tube upto 0 mark with help of long needle or long neck pipette. Avoid air bubbles in tube for accurate result. After obtaining ESR in the first hour, the tube can be spun in a centrifuge to get PCV. Wintrobe's method is more reliable when ESR is low, while Westergren's method is more sensitive for high ESR. EDTA or double oxalate is used as an anticoagulant. Length of Wintrobe tube is shorter (110 mm) and internal diameter is about 3 mm.

### Erythrocyte Sedimentation Rate by Wintrobe Method

- Males: 0-9 mm in 1 hour
- Females: 0-20 mm in 1 hour
- Children: 0-13 mm in 1 hour

### Methods used for determination of PCV

**Macrohematocrit method (Wintrobes method)**

**Microhematocrit method**

### Wintrobes method for estimation of PCV

### Apparatus required

**Wintrobes tube –**

It is 110mm long, narrow, thick walled tube with 3mm internal bore. Graduated from 0 to 10 cm with graduation on both sides in ascending and descending order on 2 sides of tube. Scale with the markings from 0 to 10 from above

downwards is used in ESR determination and from below upwards is used for PCV determination

**Pasteur pipette**

**Centrifuge**

**Procedure –**

- **2ml of venous blood is collected and mixed with double oxalate (ammonium oxalate and potassium oxalate) or EDTA powder in the proportion of 1.5mg/ml**
- **Blood is drawn into Pasteur pipette and fill in the Wintrobes tube from the bottom to 0 or 10 mark above**
- **Place the Wintrobes tube in the centrifuge machine and other Wintrobes tube filled with water on the opposite side so as to balance it.**
- **Centrifuge the tube at the speed of 3000rpm for 30 minutes**
- **After 30 minutes stop the centrifuge, take out the tube and note the readings**
- **Calculation –**
- **Hematocrit = [ Height of RBC's in mm/Height of RBC and plasma] X 100**

**Zones separated after centrifugation**

- **Top layer – Plasma (48 – 52%)**

  **Normally amber or pale yellow colour**

  **yellow – jaundice**

  **Pink or red colour indicates – hemolysis**

  **creamy white – hyperlipidemia**

  **Brown coloured – meth hemoglobinemia**

  **Cloudy (increased viscosity) – Multiple myeloma**

  **Intermediate zone – Buffy coat – Zone of platelets and leukocytes (2% – 3% or 1mm thick)**

  **Greyish – white tan layer**

  **Smears prepared from buffy coat can be used to diagnose**

  **Sub leukemic leukemia**

  - **LE cells**
  - **Detection of plasma cells**
  - **Hemoparasites**
- **Lower most zone or bottom layer – Zone of packed RBC's (45% – 50%)**
- **Normal PCV**
  - **Males – 42 -52%**
  - **Females 37 – 47%**
  - **New born – 55 – 60%**
- **Clinical implications of PCV**
- **PCV is affected by the number of RBC's, their size and plasma volume**

- **High PCV –**
  - **increased number of RBC's**
  - **Increase in size of RBC**
  - **Decrease in plasma volume**
- **Low PCV**
  - **Decrease in number of RBC's**
  - **Decrease in size of RBC**
  - **Increase in plasma volume**
- **Causes of increased PCV**
  - **Polycythemia vera**
  - **High altitudes**
  - **Hypoxia conditions**
  - **Lung and heart diseases**
  - **Dehydration**
  - **Burns (due to loss of plasma)**
- **Causes of decreased PCV**
  - **Anemia**
  - **Conditions with increased WBC's**
  - **long term illness,**
  - **infection**
  - **leukemia**
  - **Lymphoma**
  - **Hemodilution or overhydration**
  - **Acute kidney disease – lower erythropoietin production leads to less RBC's production by bone marrow**
  - **Pregnancy**

**Reticulocyte count**

**Reticulocytes** are immature red cells. They contain remnants of ribosomal RNA that was present in the cytoplasm of the nucleated precursors. Ribosomes have the property of reacting with certain dyes such as new methylene blue and brilliant cresyl blue to form a blue precipitate of granules. This reaction takes place only in unfixed vitally stained preparations. New methylene blue stains the granules and filaments in reticulocytes more uniformly than brilliant cresyl blue and is preferred. Azure blue is a good substitute for new methylene blue.

**Sample**

Venous blood collected in EDTA

**Reagents required**

New methylene blue 1g dissolved in 100ml of phosphate buffer pH6.5.

**Method**

1. Place 2-3 drops of new methylene blue in a 75x10 mm glass tube.
2. Add 2-3 drops of blood and mix.
3. Keep at 37°C for 15-20 min.

4. After resuspending the mixture, make films on glass slides as peripheral smears are made and allow to dry. The film has a greenish appearence.
5. Examine under the microscope and choose an area of the film where the cells are spread and the staining is good.
Reticulocytes are identified by the presence of dark blue granules or filaments. Count the number of reticulocytes under oil immersion seen in 1000 red cells and express as a percentage.

**Normal value**

**Adults 0.5-2.5%**

**Infants 2-5%**

**Clinical significance**

Reticulocyte count is a reflection of erythropoietic activity or the rate of production of red cells. **The count is increased in conditions with accelerated erythropoiesis as in hemolytic anemias.** The counts correlate with the severity of hemolysis.

**The reticulocyte count is low in aplastic anemia**

**Red cell indices** These indices are used in the morphological classification of anemia. They can be calculated manually. However, in most laboratories they are obtained by automated hematology analysers. The three indices are Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC)

**Mean corpuscular volume indicates the average volume of each red cell.**

$$\text{MCV} = \frac{\text{Packed cell volume}}{\text{Red cell count (millions/mm)}} X10$$

**Mean corpuscular haemoglobin indicates the average haemoglobin amount in each red cell.**

$$\text{MCH} = \frac{\text{Hemoglobin}}{\text{Red cell count (millions/mm)}} \text{X10}$$

**Mean corpuscular haemoglobin concentration is the average concentration of**
**Hb in a given volume of packed red cells.**

$$\text{MCHC} = \frac{\text{Hemoglobin}}{\text{PCV\%}} \text{X100}$$

**Reticulocyte count**

**Principle**

Reticulocytes are transitional red cells between nucleated red cells and the so-called mature erythrocytes; they are also called juvenile red cells. When stained with a supravital dye, for example New Methylene Blue, it contains stainable nucleic acids (i.e., cellular RNA). The number of Reticulocytes in the peripheral blood is a crude measurement of Erythropoietic activity. During normal red cell production, there is a controlled mechanism in place with regard to the Reticulocyte release from the bone marrow, remaining in blood circulation for a normal period of time and then finally maturing as an erythrocyte. In times of increased Erythropoietic stimuli as encountered during the different degrees of anaemia, there is a premature release of Reticulocytes into the circulation and the compromised time frame for Reticulocytes to mature in the bone marrow. The Reticulocyte count can be expressed as: % of Erythrocytes¬ Corrected % of Erythrocytes¬ Absolute count¬ Reticulocyte index (RI)¬ Reticulocyte production index (RPI)¬

Specimen Required K3EDTA blood

Test Procedure

A specimen of whole blood in EDTA is mixed by gentle inversion ten times or until the complete resuspension of cells is accomplished To a test tube (plastic or glass)

place 3 drops (approximately 150µl) of the reagent of choice e.g. New Methylene Blue stain or Azure B stain.

Add 3 drops (approximately 150µl) of patient EDTA blood to the stain solution. a) If the patient is anemic use 4 drops (approximately 200 uL) instead. b) If the patient is polycythemic use 2 drops (approximately 100 uL) instead.

Stain at ambient temperature or 37ºC between for 15 minutes.

Mix the contents of the test tube gently after the incubation period and use the stained blood to make 2 blood films. 3.6.6 Allow to air dry and then examine without fixing or counterstaining.

Reticulocyte Percentage Reticulocyte % = Number of reticulocytes counted x 100 Total no. of red cells counted Example: Counted 15 retics in 10 fields of 100 red blood cells each. Reticulocyte % = 15 x 100 1000 = 1.5%