

## MODULE

Hematology and Blood  
Bank Technique



Notes

# 8

## ERYTHROCYTE SEDIMENTATION RATE (ESR)

### 8.1 INTRODUCTION

Erythrocyte Sedimentation Rate (ESR) is the measurement after 1 hour of the sedimentation of red cells when blood is allowed to stand in an open ended glass tube mounted vertically on a stand.



### OBJECTIVES

After reading this lesson, you will be able to:

- describe the methods used for measuring ESR
- enlist the factors influencing the sedimentation of red cells
- discuss the significance of measuring ESR
- describe the reticulocyte count and its significance

### 8.2 METHODS OF MEASURING ESR

ESR can be measured in the laboratory by two methods

- Westergren method
- Automated ESR analyser

The International Council for Standardization in Hematology recommends the use of Westergren method as the standard method for measuring ESR.

#### Westergren method

The Westergren pipette is a glass pipette 30 cm in length and 2.5mm in diameter. The bore is uniform to within 5% throughout. A graduated scale in mm extends over the lower 20 cm.

## Erythrocyte Sedimentation Rate (ESR)

**Sample:** Venous blood collected in EDTA. Four volumes of blood is diluted in 1 volume of citrate. Alternatively, 2ml blood is directly collected in 0.5ml of 3.8% trisodium citrate.

### Equipment required

1. Westergren pipette
2. Stainless steel rack for holding pipette
3. Timer

### Method

1. Mix the blood sample well and draw in the Westergren pipette to the 0mm mark with a rubber teat.
2. Place the tube exactly vertical in the rack which has a rubber cork at the bottom. Fix with adjustable screws after removing the teat.
3. Leave undisturbed for exactly 60min free from vibrations and not exposed to direct sunlight.
4. Read the column of clear plasma above the column of sedimented red cells to the nearest mm.

### Normal value

Males 0-10mm 1<sup>st</sup> hour

Females 10-20mm 1<sup>st</sup> hour



**Fig. 8.1:** Westergren pipette

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**Fig. 8.2:** Westergren pipette filled with blood and placed vertically on the rubber cork in the rack

### Precautions

1. Westergren pipette must be clean and dry.
2. Dilute the blood sample just before setting up the ESR.
3. Never use mouth suction to fill the pipette.
4. The Westergren pipette must be placed vertically.
5. The ESR must be set up at room temperature (18-25°C).

### Automated method

Automated ESR analyser is a variation of the standard Westergren technique for determination of the erythrocyte sedimentation rate. The fundamental parameters are exactly the same as the Westergren method i.e. the same anticoagulant is used as the Westergren method and the ratio of blood to anticoagulant is also the same. Blood is collected in a pre-evacuated tube which is placed in the rack of the analyser. Several samples can be placed in different slots of the rack. The instrument uses a video camera and an electronic card. The video camera scans the tubes in the rack at regular intervals. These images are transferred to the microprocessing card which digitizes the images and analyses them. The system calculates the distance between the zero level of blood and plasma/cell interface as the sedimentation progresses. After multiple observations during the time of study the system mathematically calculates the ESR value which is equivalent to the value as by Westergren method at 1 hour.

### Advantages

1. The tube in which blood is collected is pre-evacuated. No manual filling of blood is required as for Westergren method.
2. It is quick and results are available in 20 minutes.
3. Data of previous samples can be stored.
4. Multiple samples can be analysed simultaneously.



Fig. 8.3: Automated ESR analyser

### Precautions

1. The instrument must be placed on a horizontal surface.
2. The surface on which the instrument is placed must be free of vibration.
3. Sample must be processed within 6 hours of collection.
4. Blood must be collected to the mark in the tube as specified by the manufacturer.
5. To enable the camera to capture images, light must travel through the tube and hence no labels must be put on the tube. The tube can be identified by numbers.

### Mechanism of sedimentation of red cells

Red cell sedimentation depends on the difference in specific gravity between red cells and plasma. As the density of red cells is more they settle down. The settling of red cells produces a settling force while the upward movement of plasma produces a retarding force. As both the forces are equal, very little settling occurs normally. When red cells form rouleaux, their mass increases and the rate of fall also increases thus increasing ESR. Rouleaux formation is limited normally by the negative charge on the red cells.

### Stages in ESR

Sedimentation occurs in **three stages**. In the **first stage**, the red cells form rouleaux. In the **second stage**, sinking of the aggregates occurs at a constant speed. In the **final stage**, the rate of sedimentation slows as the aggregated cells pack at the bottom of the tube.



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### INTEXT QUESTIONS 8.1

1. Methods of measuring ESR are ..... & .....
2. Red cell sedimentation dependence on the difference in specific gravity between ..... & .....
3. ESR is influenced when red cells form .....
4. .... facilitate rouleaux formation.

### 8.3 FACTORS AFFECTING RED CELL SEDIMENTATION

The rate of fall of red cells is influenced by a number of factors which interact with each other closely. These are

- 1. Rate of rouleaux formation:** Sedimentation of red cells is greatly influenced by the extent to which the red cells form rouleaux. More is the formation of rouleaux, higher will be the ESR. Rouleaux formation is in turn influenced by the concentration of fibrinogen and other acute phase proteins such as CRP, haptoglobin, ceruloplasmin and others. Rouleaux formation is also enhanced by immunoglobulins. All these proteins facilitate rouleaux formation and thus increase ESR. On the other hand, albumin retards rouleaux formation and ESR.
- 2. Ratio of red cells to plasma** or the hematocrit also influences ESR. In **anemic patients**, the number of red cells is less and the proportion of plasma is more. The higher proportion of plasma increases rouleaux formation and hence increases ESR.
- 3. Type of red cells** sickle cells and spherocytes do not form rouleaux and hence ESR is decreased in Hereditary spherocytosis and sickle cell anemia.
- 4. Plasma viscosity ESR** increases as the plasma viscosity increases. Viscosity is dependent on the concentration of fibrinogen and other plasma proteins.
- 5. Length of the tube:** Longer the tube, longer will be the duration of the second phase and higher will be the ESR.
- 6. Verticality of the tube:** If the tube is not vertical, sedimentation occurs more quickly due to streaming of blood down the wall of the sloped tube.
- 7. Temperature** sedimentation is accelerated as the temperature increases. ESR is hence always measured at room temperature(18-25°C).



**INTEXT QUESTIONS 8.2**

1. ESR increases when plasma viscosity .....
2. ESR is increased when plasma is .....
3. In sickle cell anemia, the ESR is .....
4. Sedimentation is accelerated when temperature .....

**8.4 SIGNIFICANCE OF MEASURING ESR**

ESR is a useful screening test for the presence of an underlying acute phase response. Most acute and chronic infections, neoplastic diseases are associated with alteration in plasma proteins and increased ESR. In patients with Tuberculosis, it provides an index of disease activity and of the response to therapy. Similarly in Rheumatoid arthritis, ESR is an index of activity. It is useful in the diagnosis of temporal arteritis and polymyalgia rheumatica.

**Causes of elevated ESR**

1. Tuberculosis
2. Rheumatoid arthritis
3. Myocardial infarction
4. Multiple myeloma and other plasma cell disorders
5. Pregnancy
6. Neoplastic diseases
7. Degenerative diseases

**Causes of reduced ESR**

1. Polycythaemia
2. Hypofibrinogenemia
3. Hereditary spherocytosis
4. Congestive heart failure
5. Sickle cell anemia

**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



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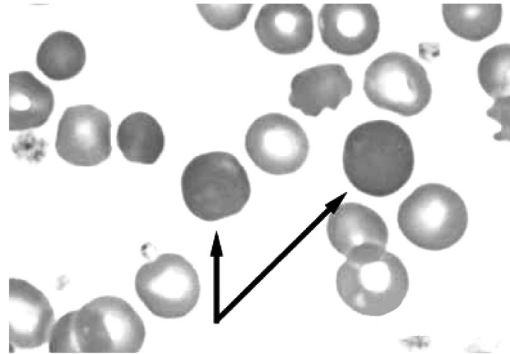


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### Erythrocyte Sedimentation Rate (ESR)

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.

If a blood film is fixed in methanol, reticulocytes appear as diffusely basophilic cells and are called **polychromatophils**.



**Fig. 8.4:** Basophilic red cells or polychromatophils as seen on peripheral smear

#### Method

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 75x10mm 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 appearance

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5. Examine under the microscope and choose an area of the film where the cells are spread and the staining is good. Red cells have a bluish hue while 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.

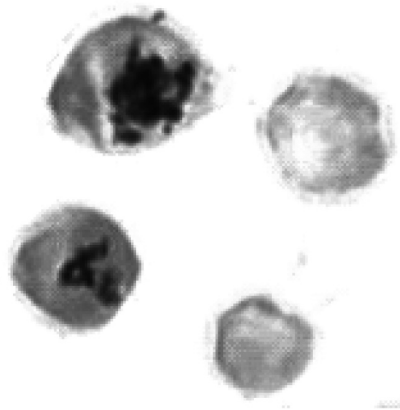


Fig. 8.5: Reticulocytes with granules

### Normal value

Adults 0.5-2.5%

Infants 2-5%

### Significance of reticulocyte count

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.

In nutritional anemias, the count is low. On treatment with hematinics the count rises. Hence, reticulocyte count is used to **assess response to hematinic therapy** in these patients.

The reticulocyte count is **low** in aplastic anemia and red cell aplasia. When aplastic crisis occurs in hemolytic anemia the reticulocyte count is low.



### WHAT HAVE YOU LEARNT

- ESR is the measurement after 1 hour of the sedimentation of red cells when blood is allowed to stand in an open ended glass tube mounted vertically

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on a stand. It can be measured in the laboratory by Westergren method or by an automated analyser. Several factors influence ESR including concentration of fibrinogen and other acute phase proteins. ESR is a screening test for acute phase response and is elevated in acute and chronic infections, degenerative and neoplastic diseases.

- Reticulocytes are precursors of red cells which have remnants of ribosomal RNA. This stains with dyes such as new methylene blue to form blue precipitates or granules. On blood films fixed with methanol, reticulocytes appear as basophilic cells called polychromatophils. Reticulocyte count is a reflection of erythropoietic activity.



### TERMINAL QUESTIONS

1. What is the standard method for measuring ESR
2. Name two conditions in which ESR is elevated.
3. Name two conditions in which ESR is reduced.
4. Name two dyes used for reticulocyte count.



### ANSWER TO INTEXT QUESTIONS

#### 8.1

1. Western method and automated ESR analyser
2. Red cells and plasma
3. Rouleaux formation
4. Proteins

#### 8.2

1. Increases
2. High
3. Decreased
4. Increases