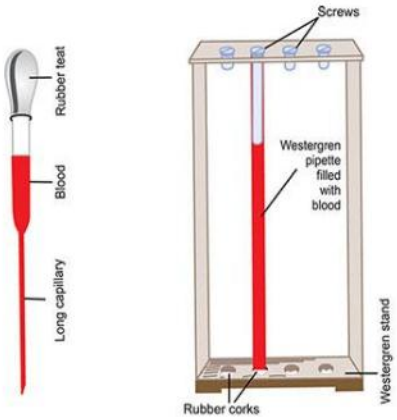
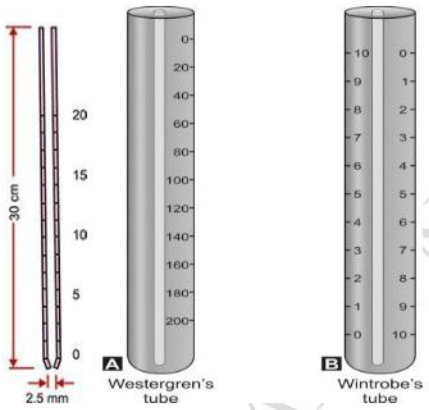
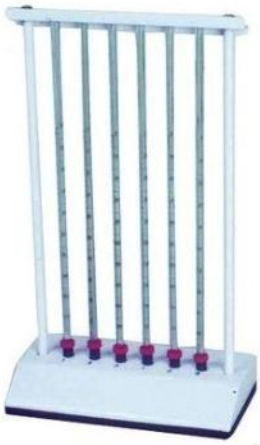
EXPERIMENT NO.: 5. b **DATE:**

AIM: TO DETERMINE ERYTHROCYTE SEDIMENTATION RATE BY WESTERGREN'S METHOD







Westergren

Wintrobe

WORKING PRINCIPLE:

When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed 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). This mechanism involves three stages:

1. **Stage of aggregation:** It is the initial stage in which piling up of RBCs takes place. The phenomenon is known as Roulcaux formation. It occurs in the first 10-15 minutes.
2. **Stage of sedimentation:** It is the stage of actual falling of RBCs in which sedimentation occurs at constant rate. This occurs in 30-40 minutes out of 1 hour, depending upon the length of the tube used.

3. **Stage of packing:** This is the final stage and is also known as stationary phase. In this, there is a slower rate of falling during which packing of sedimented RBCs in column occurs due to overcrowding. It occurs in final 10 minutes in 1 hour.

THEORY:

- The erythrocyte sedimentation rate (ESR) is a common hematological test for nonspecific detection of inflammation that may be caused by infection, some cancers and certain autoimmune diseases. It can be defined as the rate at which Red Blood Cells (RBCs) sediment in a period of one hour.
- When anticoagulated blood is allowed to stand undisturbed for a period of time, the erythrocytes tend to sink to the bottom.
- Two layers are formed, the upper plasma layer and the lower one of red blood cells.
- The rate at which the red cells fall is known as the erythrocyte sedimentation rate.
- The first is the stage of aggregation when the red cells form rouleaux (RBCs cling together like coins in pile).
- This is followed by the stage of sedimentation in which the falling of the red cells takes place.
- The rate of falling of erythrocyte is directly proportional to the aggregation in first stage.

Clinical significance

- ESR is increased in all conditions where there is tissue breakdown or where there is entry of foreign proteins in the blood, except for localized mild infections.
- The determination is useful to check the progress of the infectious disease. If the patient is improving the ESR tends to fall.
- If the patient's condition is getting worse the ESR tends to rise. The ESR increases high in some chronic bacterial diseases like tuberculosis, typhoid, rheumatic diseases etc.

NORMAL RANGE:

- **Male:** 0-15 mm after 1st hour.
- **Female:** 0-20 mm after 1st hour.

FACTORS AFFECTING ESR:

i. The changed levels of plasma proteins such as fibrinogen and globulins tend to increase rouleaux formation. ESR is therefore increased in any condition causing an increase in fibrinogen (any cause of tissue breakdown such as tuberculosis and other chronic infections) or globulins (rheumatic fever, myeloma, kala-azar, etc.)

ii. Albumin retards sedimentation.

iii. High blood count however, tend to lower the sedimentation rate, while low blood counts tend to accelerate the rate of fall.

iv. ESR is greater in women than in men.

v. During pregnancy ESR gradually increases after 3rd month and returns to normal in about 3 to 4 weeks after delivery.

vi. ESR is low in infants and gradually increases up to puberty.

The Laboratory factors which influence ESR are as follows:

- **Time:** The test should be performed as early as possible after the collection of fasting specimen. There is progressive decrease in sedimentation in first four hours and after that there is a rapid decrease in sedimentation.
- **The length of the ESR tube:** ESR is greater with longer tubes (Westergren's tube) than with shorter tube (Wintrobe's tube). To ensure reliable results the column of blood should be as high as possible. The internal diameter of the tube should be more than 2.5 mm. The tubes should be kept in vertical position. Deviation of the tubes from the vertical position increases the ESR.
- **Temperature:** The red cell sedimentation is increased at higher temperature.

METHODS FOR ESR DETERMINATION:

There are two main methods to determine ESR :

1. Wintrobe's method
2. Westergren's method

Each method produces slightly different results. Mosely and Bull (1991) concluded that Wintrobe's method is more sensitive when the ESR is low, whereas, when the ESR is high, the Westergren's method is preferably an indication of patient's clinical state.

PROCEDURE

1. Westergren's method

Requirements:

- Anticoagulated blood (0.4 ml of 3.13% trisodium citrate solution + 1.6 ml blood)
- Westergren tube
- Westergren stand
- Rubber bulb (sucker)

It is better method than Wintrobe's method. The reading obtain is magnified as the column is lengtheir. The Westregren tube is open at both ends. It is 30 cm in length and 2.5 mm in diameter. The lower 20 cm are marked with 0 at the top and 200 at the buttom. It contains about 2 ml of blood.

1. Fill the Westergren's tube exactly up to zero mark by means of a rubber bulb (avoid air bubbles).
2. Place the tube upright in the stand. It should fit evenly into the groove of the stand.
3. Note the time. Allow the tube to stand for exactly one hour.
4. Exactly after one hour, note the level to which the red cell column has fallen.
5. Report the result in terms of mm/after 1st hour.

Normal values

For males: 0-10 mm/hr

For females: 0-15 mm/hr

2. Wintrobe method:

Requirements:

- Anticoagulated blood (EDTA, double oxalate)
- Pasteur pipette
- Timer
- Wintrobe's tube
- Wintrobe's stand

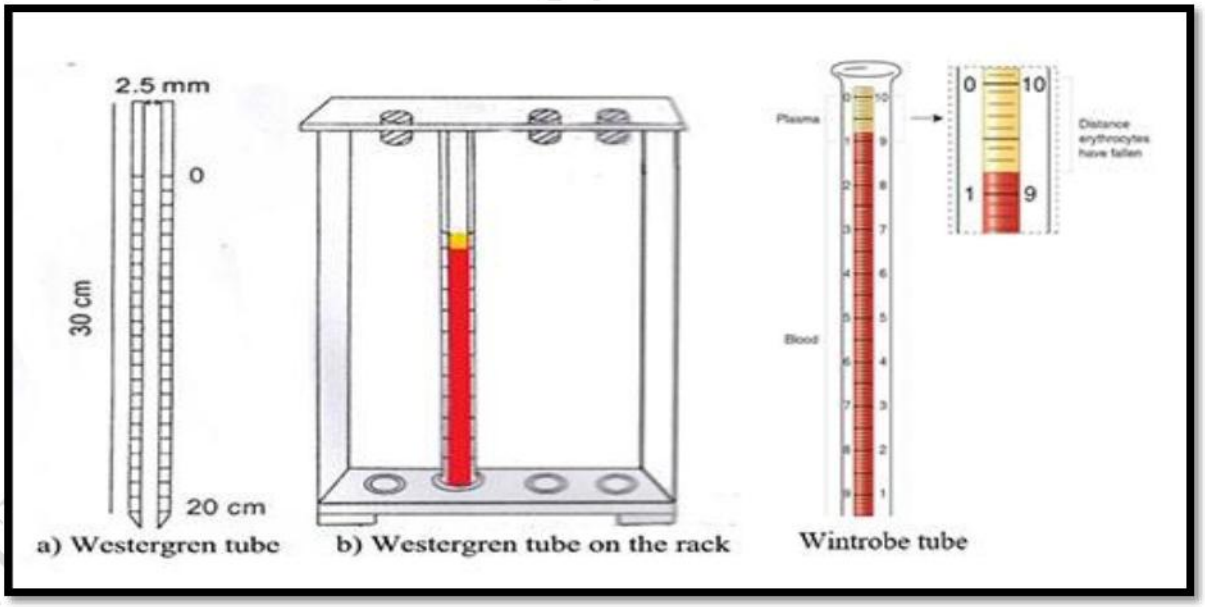
This method uses Wintrobe's tube, a narrow glass tube closed at the lower end only. The Wintrobe's tube has a length of 11 cm and internal diameter of 2.5 mm. It contains 0.7-1 ml of blood. The lower 10 cm are in cm and mm. The marking is 0 at the top and 10 at the bottom for ESR

1. The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long.
2. 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.
3. The shorter column makes this method less sensitive than the Westergren method because the maximal possible abnormal value is lower.
4. Fill the Wintrobe tube exactly up to zero mark by means of a rubber bulb (avoid air bubbles).
3. Place the tube upright in the stand. It should fit evenly into the groove of the stand.
4. Note the time. Allow the tube to stand for exactly one hour.
5. Exactly after one hour, note the level to which the red cell column has fallen.
6. Report the result in terms of mm/after 1st hour.

Normal values :

For males: 0-9 mm/hr

For females: 0-20 mm/hr



CLINICAL SIGNIFICANCE OF ESR

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein. The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.

CAUSES OF A SIGNIFICANTLY RAISED ESR :

- All types of anemias except sickle cell anemia
- Acute and chronic inflammatory conditions and infections including:
  - HIV disease
  - Tuberculosis
  - Acute viral hepatitis
  - Arthritis
  - Bacterial endocarditis
  - Pelvic inflammatory disease
  - Ruptured ectopic pregnancy
  - Systemic lupus erythematosus
- African trypanosomiasis (rises rapidly)
- Visceral leishmaniasis
- Myelomatosis, lymphoma, Hodgkins disease, some tumours
- Drugs, including oral contraceptives

CAUSES OF REDUCED ESR :

- Polycythaemia
- Poikilocytosis
- Newborn infants
- Dehydration
- Dengue haemorrhagic fever
- Other conditions associated with haemoconcentration

OBSERVATION TABLE

Method	At the end of one hr		At the end of two hr	
	Male	Female	Male	Female
Westerngren				
Wintrobe				

CONCLUSION

- The ESR of the given sample is normal / abnormal
- If abnormal then is it higher/lower than normal.

SIGNATURE OF TEACHER