CLS 291 Clinical Hematology 1

جامعة الملك سعود
King Saud University

Lecture 3

Packed Cell Volume (PCV) & Erythrocytes Sedimentation Rate (ESR)

I. Packed Cell Volume (PCV)

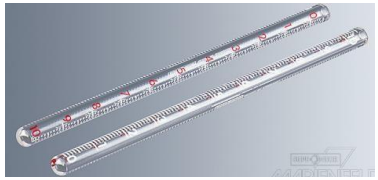
1. Packed Cell Volume (PCV)

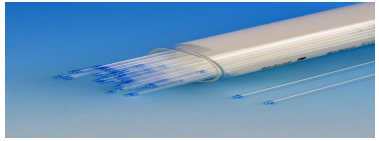
- The packed cell volume (PCV) is a measurement of the ratio of the volume occupied by the RBCs to the volume of whole blood in a sample of capillary or venous blood.

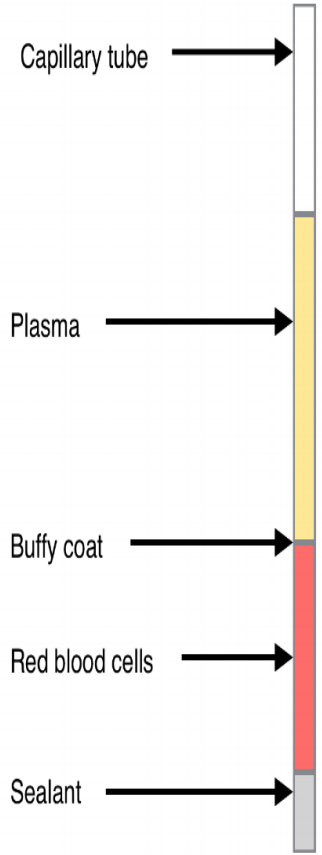
- **Clinical significance:**
  - Used as a simple screening test for anemia, polycythemia, hemodilution, or hemoconcentration.

- **Application:**
  - Used In conjunction with an RBCs count to calculate the mean cell volume **(MCV).**
  - Used in conjunction with the hemoglobin concentration to calculate the mean cell hemoglobin concentration **(MCHC).**
  - Used as a rough guide to **measuring Hb.**

- **Methods:**
  1. Wintrob's method,
  2. Microhaematocrit method
  3. Electronic method – in automated instrument (as Hct in CBC report).

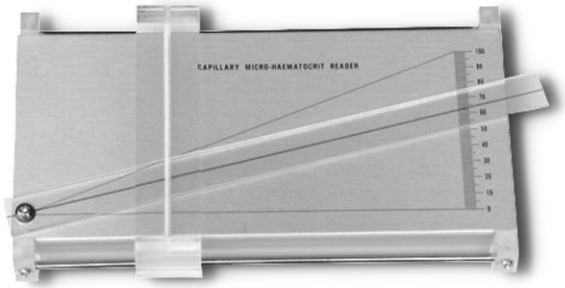


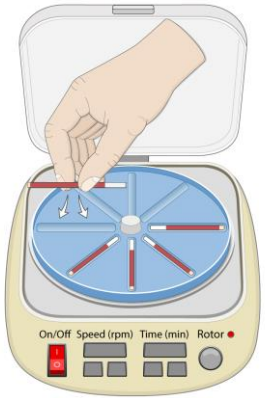


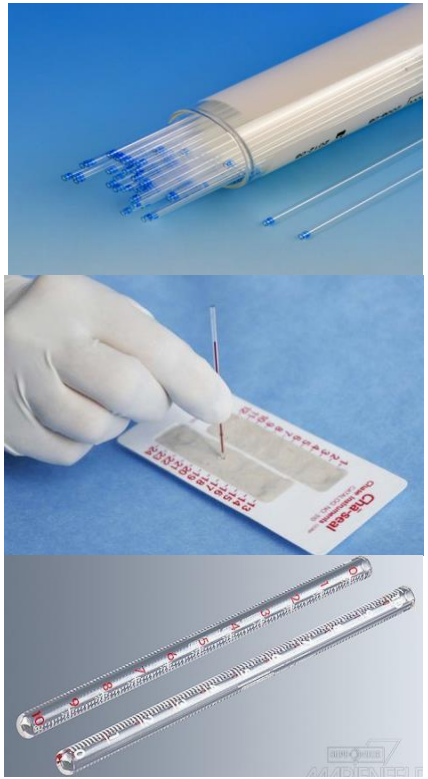


Microhaematocrit Materials

- **Materials:**
  - Capillary tube.
  - Clay.
  - Centrifuge.
  - PCV reader.
  - EDTA whole blood sample.







Microhaematocrit procedure

- Switch on the centrifuge.
- Open the lid.

- Unscrew the metal cover.

- Check that the centrifuge is clean and free from debris or previous samples.

Microhaematocrit procedure

- Gently invert the sample tube to mix the sample.
- Hold the tube at an angle and introduce the microhaematocrit (capillary) tube.
- **Allow blood to track up the tube.**
- Continue until the tube is about 3/4 full.

- Put your index finger over the top of the capillary tube before removing it from the sample or blood will leak!
- Keeping your finger over the end of the tube, wipe the outside of the capillary tube clean with a piece of tissue.

- Seal one end ONLY with the clay by placing the capillary tube onto the plasticine, then remove your index finger from the top.
- Having removed your finger then, gently press the capillary tube into the plasticine and then withdraw the tube.
- Wipe the tube clean with a tissue.

Microhaematocrit procedure

- Check there is a sufficient plasticine plug.
- If not, then discard the capillary tube into a sharps bin and start again.

- Fill 2 tubes and place them opposite each other in the centrifuge.
- This is to **balance** the centrifuge; every tube must have another tube opposite it.
- Place the plasticine plug end of the tube **against the rubber (outer) edge** to stop the contents from spilling out when spun.

- Screw the metal cover (plate) on firmly.
- Close and secure the centrifuge lid.
- **Centrifuges are dangerous if used incorrectly so ensure that steps 8 & 9 are followed carefully.**
- Centrifuge for 5 min at 4000RPM

Microhaematocrit reading

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Set the time to 5 minutes then start the centrifuge spinning. Once the centrifuge has come to a complete stop, open the lid and remove the metal cover (plate).

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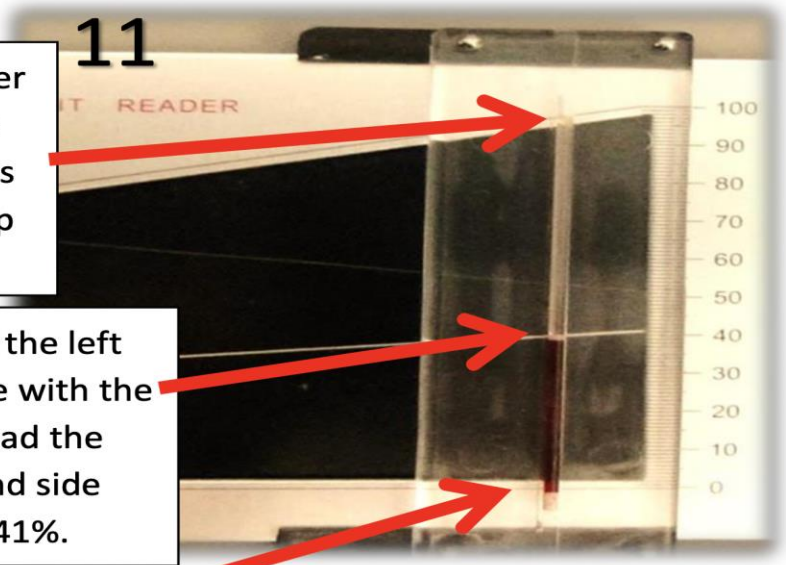


3) Move the slider so the top of the plasma fraction is level with the top line (100%).

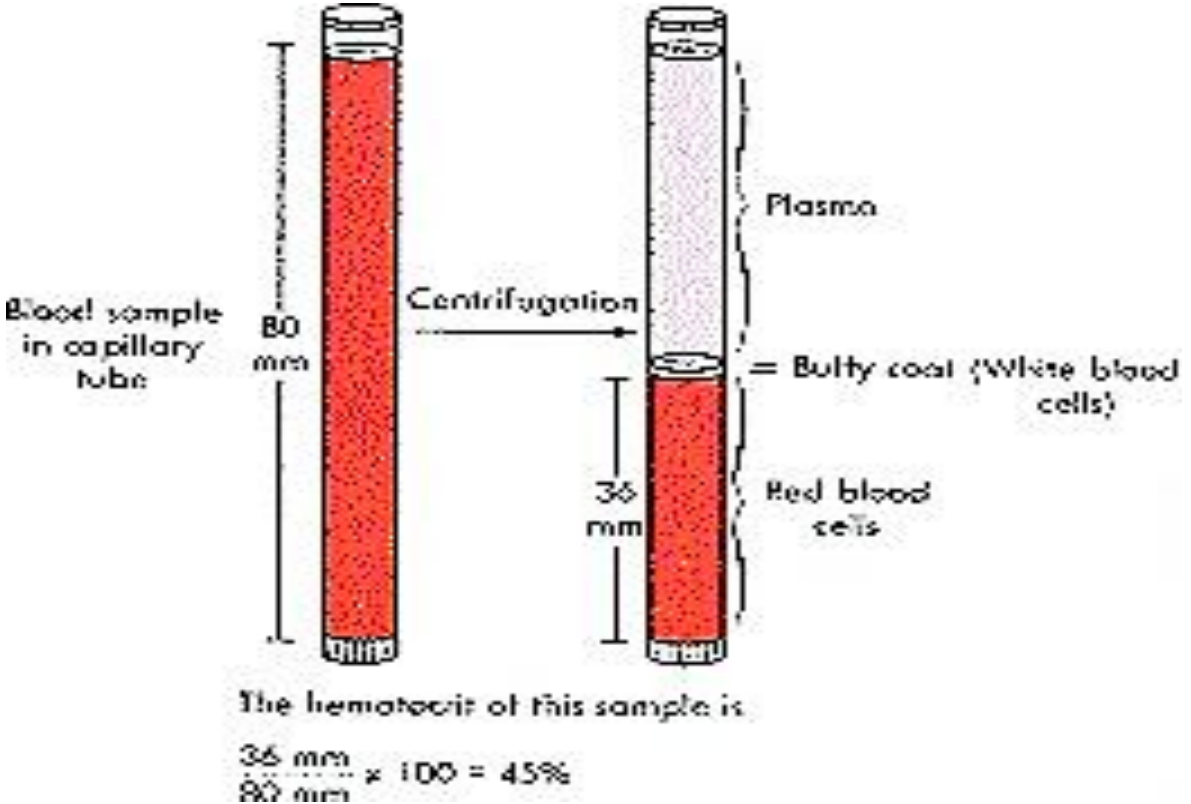
4) Use the adjuster on the left to align the middle line with the top of the red cells. Read the PCV from the right hand side scale. In this case it is 41%.

2) Adjust the tube on the slider so that the top of the plasticine is level with the bottom line (0%).

1) Place the capillary tube onto the haematocrit reader. It should be positioned as shown above.

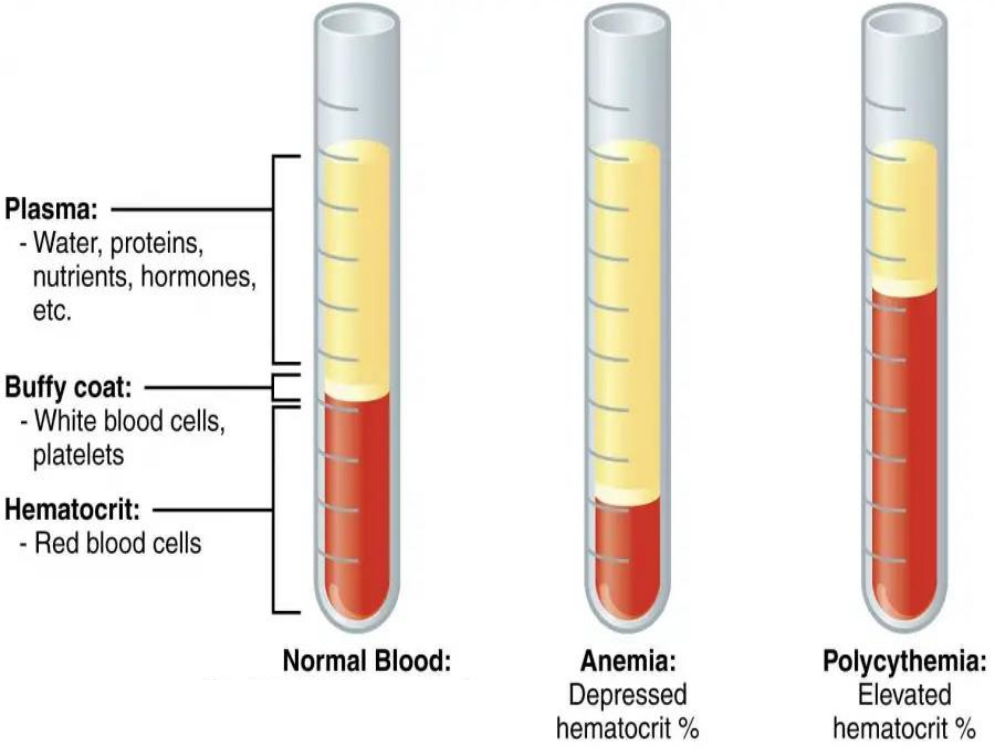
- The test should be read as soon as possible after centrifugation because the red cells begin to swell and the interface becomes progressively more indistinct.
- White cells and platelets (the buffy coat) must be excluded as far as possible from the reading of the PCV.

Microhaematocrit



Result Interpretation

- Packed cell volume (PCV) or haematocrit (Hct) normal range:
  - Men 0.45 ± 0.05 l/l or 45 % ± 5%
  - Women 0.41 ± 0.05 l/l or 41 % ± 5%
- High PCV indicates **polycythemia**.
- Low PCV indicates **anemia**.



II. Erythrocytes Sedimentation Rate (ESR)

Erythrocytes Sedimentation Rate

- **The ESR test** measures the rate at which the red blood cells (RBCs) in a sample of whole blood fall to the bottom of the Westergren tube.
- This process of "falling" is called **sedimentation**.
- **Clinical significance:** ESR is a common screening (not specific) hematology test that may indicate and monitor an increase in **inflammatory activity** within the body.
- **Advantages of the ESR test:**
  1. Reproducibility.
  2. Low cost.

Erythrocytes Sedimentation Rate

There are different methodologies used to test ESR:

a. **Manual:**

1. Westergren method (Reference method)
   - Reproducible.
   - Cheap.
   - Time-consuming.

2. Wintrobe method (misleading results)

b. **Automated method:** e.g., Ali Fax
   - Decrease the biohazardous risk to operators.
   - Decrease the time (takes only 20 seconds).
   - Not reliable.
   - Needs confirmation.

Westergren method

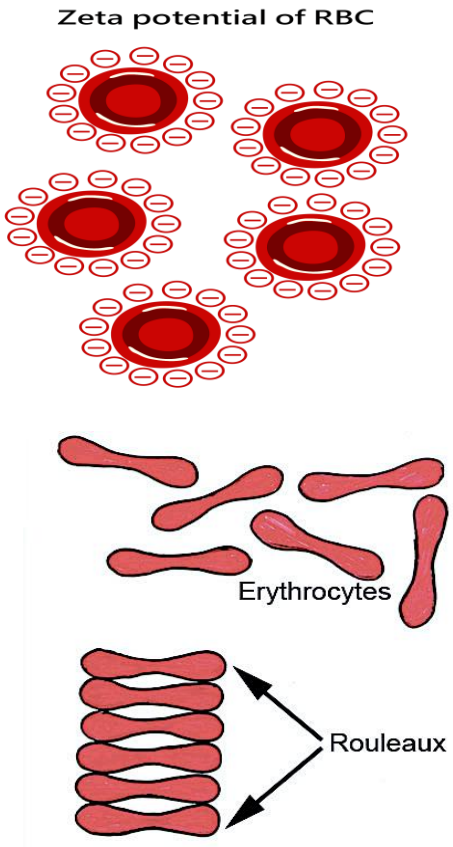
Ali Fax

ESR Mechanism

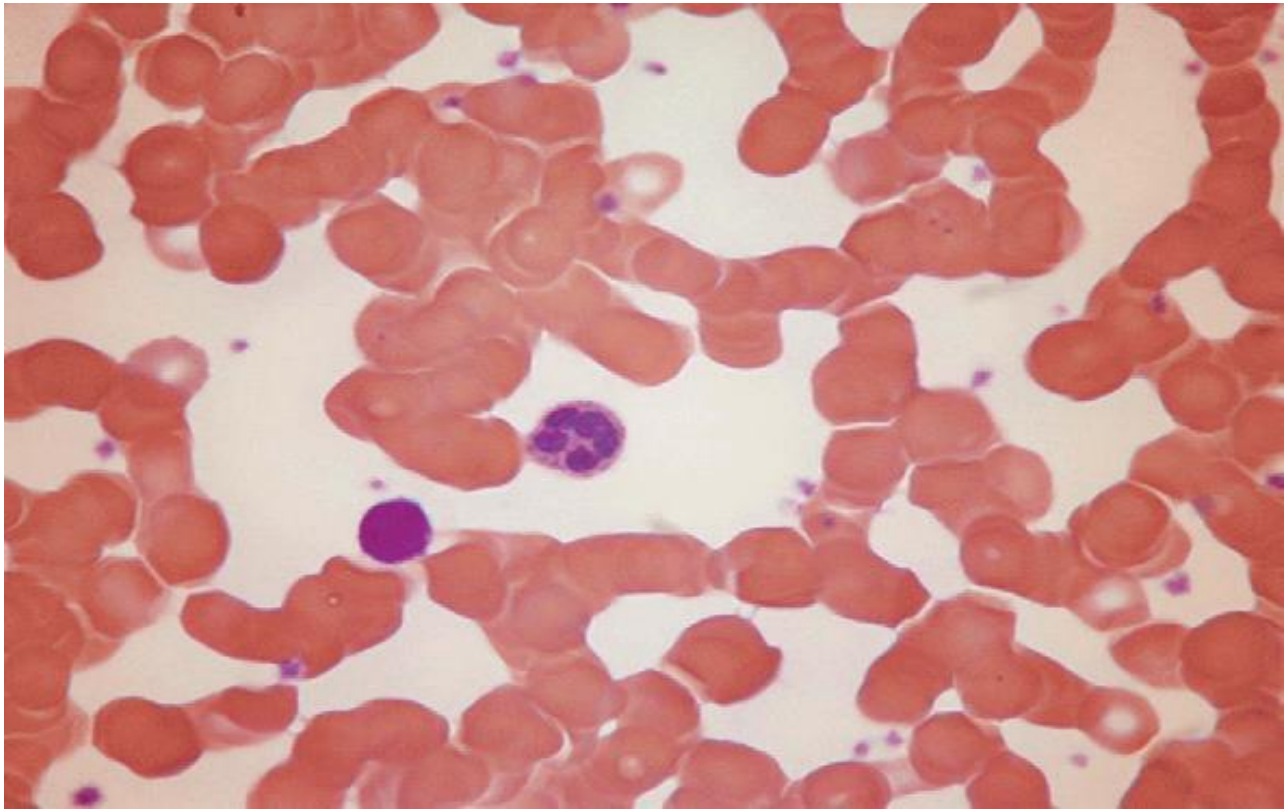
- Normally, **RBCs** have negative charges on the outside of the cells, which cause them to repel each other.

- Many **plasma proteins** have positive charges and can effectively neutralize the negative surface charges of the RBCs, which allows for the to clump together and thus form **rouleaux**.

- When a group of RBCs are clumped together will form a stack (similar to a stack of coins) called a **rouleau (pleural is rouleaux).**



Microscopic Image of Rouleaux



Specimen Requirements and Procedure

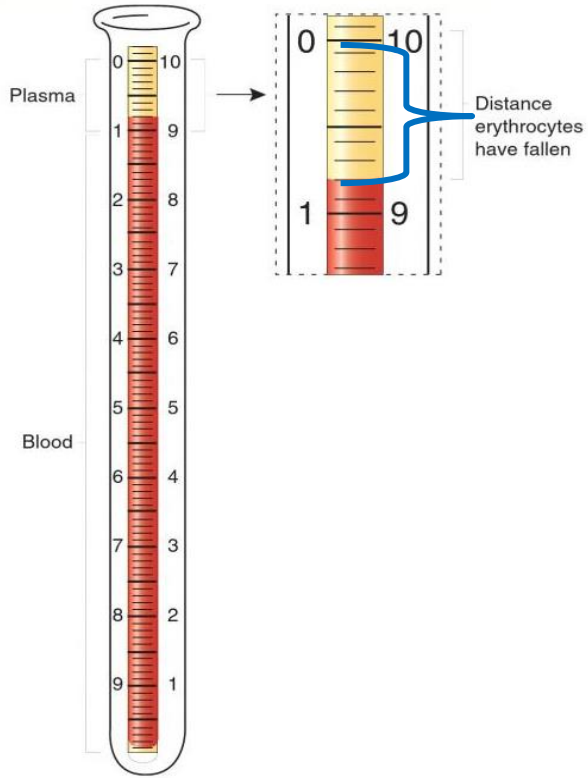
- **Materials:**

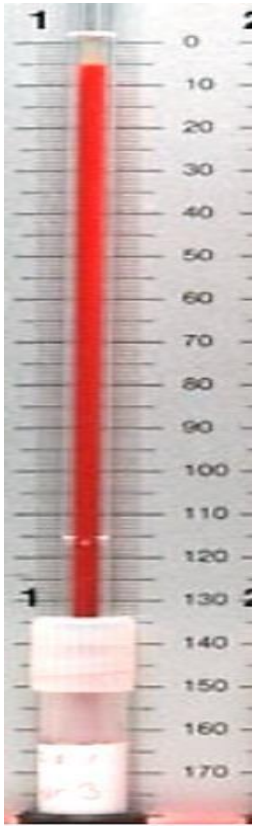
1. EDTA blood sample
2. Pipette
3. 3.8% trisodium citrate.
4. Westergren tube.
5. Rack

- **Westergren procedure:**

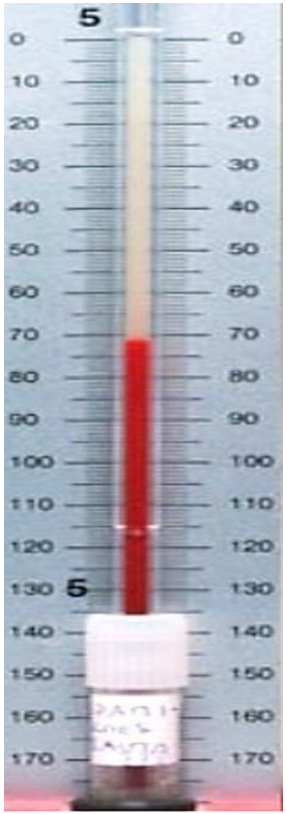
1. Add 2 (or 1.75) ml of EDTA blood into a tube containing 0.5 ml 3.8% trisodium citrate, and mix by inverting the tube.
   - Note: The ratio of trisodium citrate to blood is 1:4.
2. Fill the Westergren tube to the zero mark.
3. Place the Westergren tube exactly in a vertical position at RT.
4. After 1 h, read the distance from the 0 mark to the top of the RBC column. Record the result as **mm/h.**

ESR Result Reading and Interpretation

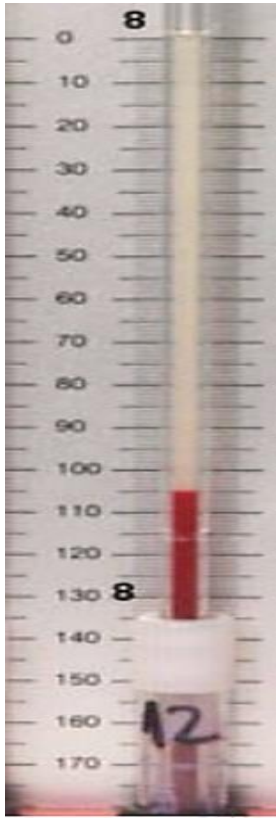




5 mm/h



70 mm/h



105 mm/h

Erythrocytes Sedimentation Rate

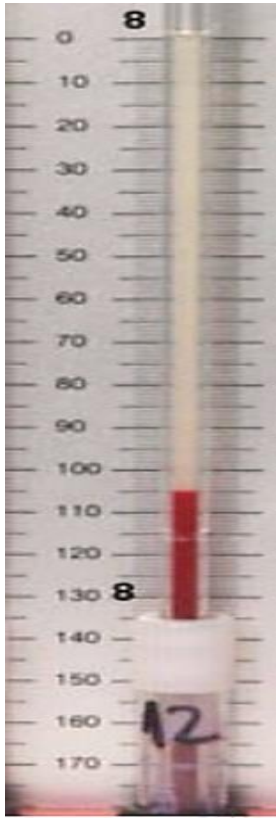
- **Normal ranges:**
  - **Men:**
    - < 70 years less than 14 mm/hour
    - >70 years less than 30 mm/hour
  - **Women:**
    - < 70 years less than 20 mm/hour
    - >70 years less than 35 mm/hour
  - **Children:**
    - Less than 10 mm/ hour

Increase ESR causes

- **Physiological condition:**
  - Female
  - Pregnancy
  - Age

- **Pathological conditions:**
  - High fibrinogen level: proteins concentration (acute phase proteins) such as globulin, fibrinogen (increase)---< Rouleaux ---< ESR. E.g. inflammation, infection, autoimmune disorders
  - RBC abnormalities: affecting RBC charge (-ve)

- **Technical factors:** high temperature



Deceased ESR causes

- **Pathological conditions:**
  - Low fibrinogen level: hypofibrinogenemia
  - RBC abnormalities: polycythemia, sickle cell anemia, Spherocytosis.
- **Technical factors:** clotted sample