Chapter 5

Erythrocyte Sedimentation Rate (ESR) and Packed Cell Volume (PCV)

ERYTHROCYTE SEDIMENTATION RATE

The erythrocyte sedimentation rate (ESR) is the rate at which red blood cells sediment in a period of one hour.

It is also known as sedimentation rate (sed rate).

Brief History of ESR

This test was invented by the Polish pathologist Edmund Biernacki in 1897. In some regions of the world, the test continues to be referred to as Biernacki's reaction. In the year 1918, the Swedish pathologist Robert Sanno Fahraeus along with Alf Vilhelm Albertsson Westergren described the same and is eponymously remembered as Fahraeus-Westergren test. But in the UK, it is usually termed Westergren test which became popular throughout world.

Mechanism of Erythrocyte Sedimentation

The ESR or rate of fall of red cells is governed by the balance between pro-sedimentation factors, mainly fibrinogen and those factors resisting sedimentation, e.g. the negative charge of the erythrocyte (zeta potential). The decreased zeta potential promotes rouleaux formation and hence raised ESR.

ESR depends upon the difference in specific gravity between red cells and plasma. Also, it is greatly influenced by the extent to which RBCs form rouleaux, which sediment stacks of RBCs than single cell. Many other factors are also responsible which include the ratio of RBCs to plasma (i.e. PCV), the plasma viscosity, the bore of the tube, dilution, if any of the blood, the verticality of ESR tube, etc.

The very important factor is rouleaux formation and red cell clumping. This is mainly controlled by the plasma concentration of fibrinogen and other acute phase proteins/reactants, e.g. haptoglobin, ceruloplasmin, α_1-antitrypsin and C reactive protein (CRP). Rouleaux formation is also enhanced by increased concentration of plasma immunoglobulin. On the contrary, it is retarded by higher concentration of albumin and test done with defibrinated blood (≤1 mm/hr), which removes fibrinogen. In anaemia, there may be quantitative deficiency of RBCs, so ratio of RBCs to plasma is altered which results more rouleaux formation and increased ESR.

Methods of ESR Estimation

i. Westergren method
ii. Wintrobe method
iii. Landau method: Not accurate, used in the past in children with limited supply of blood. The method uses capillary blood from heel, toe or fingertip.

iv. Electronic method by automated analyzers.

The first two methods are commonly used methods which are done by manual technique. Of these two, Westergren method is most popular and commonly used method.

Westergren Method using Westergren Pipette (Fig. 5.1)

Though the equipment is sometimes called Westergren tube, it should be called pipette (more scientific term) as it is open at both ends.

Westergren pipette is a slender, thick-walled pipette. It is 300 mm long (i.e. 30 cm or 12 inches) of which lower 200 mm is graduated (markings) and upper 100 mm part is ungraduated. The inner diameter of pipette should not be less than 2.5 mm. The capacity of the tube is about 1 ml.

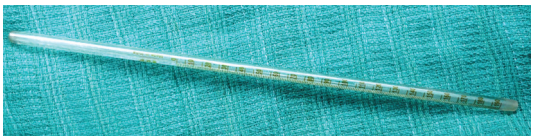

Fig. 5.1: Westergren pipette

Anticoagulant used: 3.8% trisodium citrate solution (no other anticoagulant with this method), 109 mmol/L (32 gm/L, $Na_3Ca_6H_5O_7.2H_2O$). EDTA mixed blood is used in modified Westergren method.

Blood and anticoagulant ratio: For this test 2 ml of venous blood is mixed with 0.5 ml of sodium citrate anticoagulant. So, the blood and anticoagulant ratio is 4:1.

Method or procedure: 0.5 ml of 3.8% sodium citrate solution is taken in a test tube. Then, 2 ml of venous blood (usually from antecubital vein) is mixed with the anticoagulant immediately with the help of a syringe. The sample is well mixed.

The anticoagulated blood is drawn up in to Westergren pipette up to 200 mm mark with the help of a teat or mechanical device (mouth suction is avoided). The pipette is now set exactly vertical in a Westergren stand (Fig. 5.2). A spring clip, pressing on the top and rubber piece at the lower end hold the pipette in the Westergren stand or rack. Now the pipette in the rack which is vertically placed is kept at room temperature without vibration and exposure to sunlight.

Modified Westergren method: It produces the same results but uses EDTA blood rather than citrate as an anticoagulant. In this method, 2 ml of EDTA blood is diluted with 0.5 ml of 0.85% sodium chloride or with 0.5 ml of 3.8% sodium citrate. Precision is poor when EDTA blood is undiluted.

This method has the advantage that same EDTA-mixed blood may be used for other haematologic studies.

Recording result: The RBCs begin to settle down and a clear plasma zone is formed above the settled RBCs. The upper level of the RBC column is read (zero mark is upside and 200 mark is downside) at the end of one hour.

The measurement in mm is ESR (Westergren, 1 hour).

Previously it was thought that mean average ESR per hour (total duration 2 hours) is more accurate.

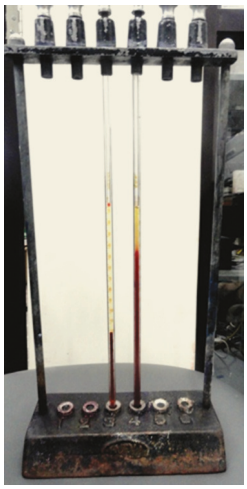

Fig. 5.2: Westergren or ESR stand (rack)

Mean ESR = (1st hour result + ½ of 2 hours result) ÷ 2

But later on, concept of mean ESR disregarded because result after 1 hour gives more accurate result.

Normal range of ESR: The ESR gradually increases with age. Westergren's original upper limit of normal (10 mm/hour for men and 20 mm/hour for women) seems to be low. According to studies by scientists, upper limits of reference values in this method should be as follows:

	Men	Women
i. Below age 50 years	0–15 mm/hr	20 mm/hr
ii. Above age 50 years	0–20 mm/hr	30 mm/hr
iii. Above age 85 years	0–30 mm/hr	42 mm/hr

But in Indian context, acceptable normal range for ESR is

- Men: 4–14 mm/hr
- Women: 6–20 mm/hr
- Newborn: 0–2 mm/hr
- Newborn to puberty: 3–13 mm/hr

Wintrobe's Method

Instrument: Wintrobe's tube is a special, thick-walled glass tube 11 cm long with an internal diameter of 3 mm and the bottom 10 cm are graduated. It has flat inner base. It is calibrated at 1 mm intervals to 100 mm and holds about 1 ml of blood. Graduations are from zero (top) to hundred (bottom) for ESR and zero (bottom) to hundred (top) for PCV.

Anticoagulant: Venous blood is mixed with EDTA (preferred), or double oxalate or heparin.

Methods

1. 2 ml venous blood is collected and mixed with anticoagulant (EDTA) in the vial immediately.
2. Wintrobe tube is filled with this blood till zero mark on top with the help of a Pasteur pipette.
3. The tube is now kept vertically over stand for one hour and is noted by measuring the free plasmatic zone above (descending orders, 0 marks on top and 100 marks on bottom).

Normal range: Men 0–9 mm/hr, women 2–20/hr.

Stages of Sedimentation (ESR)

Stage 1. Rouleaux formation: In this stage (first 15 minutes) red cells form rouleaux and minimum sedimentation occurs.

Stage 2. Formation of fine threads by proteins: During this stage (second 15 minutes) fibrinogen and globulin in plasma form network by forming fine threads. The rouleaux of red cells are trapped within this network and becomes heavier. So, they begin to fall (settling) rapidly.

Stage 3. Rapid fall of protein network: In this phase (third 15 minutes), red cell mass and protein network fall rapidly.

Stage 4. Packing of red cells: In the last 15 minutes, the sedimented red cell mass—protein undergoes packing at the bottom of pipette.

MODIFIED WESTERGREN METHOD

Disposable ESR Pipette (Westergren Method)

A disposable ESR pipette offers a highly accurate, risk free, easy, efficient and safe method for performing *Westergren's ESR determination* directly in a 12 × 75 mm/13 × 75 mm non-vacuum/vacuum blood collection tube (Fig. 5.3A and B).

Disposable ESR pipette is made of clear polystyrene, which gives a clear visibility of the blood for determining ESR. A biodegradable vacuum plug at the bottom,

which creates pressure on the blood in the 12 × 75 mm non-vacuum or vacuum blood collection tubes and thus the blood raises up in the pipette till the zero level mark, which acts as a barrier that stops hazardous substances (blood) from escaping through the top of the pipette.

Advantages of a Disposable ESR Pipette

1. A single time use of the pipette.
2. It avoids mouth pipetting and ensures user safety.
3. Can be used with almost all tubes like 12 × 75 mm/13 × 75 mm Non-vacuum/vacuum tubes.
4. Highly accurate, reproducible, risk free, easy, efficient and safe method of conducting an ESR.
5. Fibrous barrier protects user from hazardous aerosols.
6. Speed of application.

Procedure

1. Collect 1.6 ml of whole blood in 0.4 ml of sodium citrate 3.8% solution or take 1.6 ml of EDTA anti-coagulated whole blood in 0.4 ml of saline (use 12 × 75 mm non-vacuum blood collection tubes with 0.4 ml of sodium citrate 3.8% solution).
2. Mix the blood gently.
3. Gently insert the lower end of pipette bearing the vacuum plug in to the blood. Collect ion of tube and using continuous force, push the pipette down to the bottom of the blood collection tube.
4. The blood will automatically rise into the pipette and stop at the "zero" mark.
5. Place the assembly (tube + pipette absolutely vertical on a suitable stand (example rack for ESR pipette). And allow the blood cells to sediment without disturbing it for 60 minutes.
6. At the end of 60 minutes the numerical results are read in millimeters directly from the imprinted scale on the pipette.

Errors Faced while Using a Disposable Pipette

1. If the blood collection tube is cracked
2. If the ESR pipette has not been placed absolutely vertical
3. If the blood collection tube is not gently mixed
4. If the proportion of the blood and sodium citrate/EDTA is improper
5. If the disposable ESR pipette is reused.

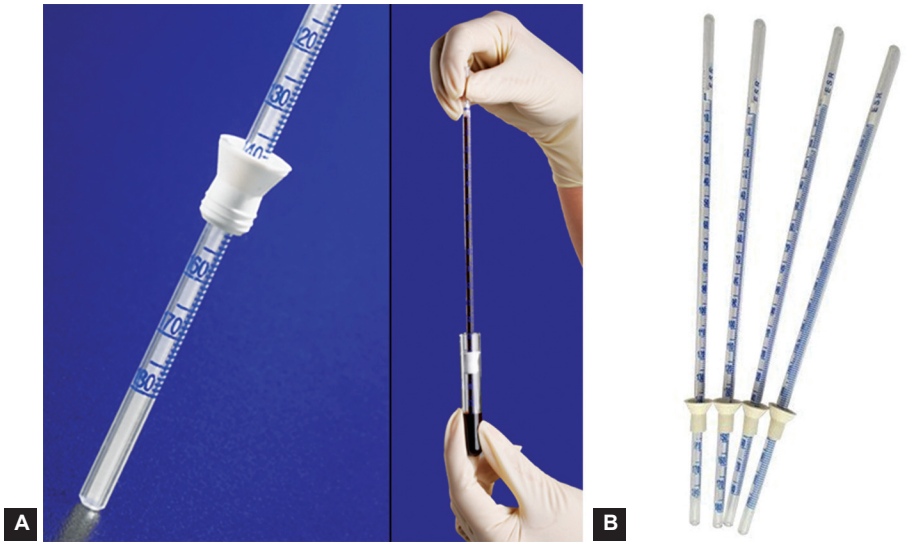

Fig. 5.3A and B: ESR estimation with disposable plastic pipettes (modified Westergren method)

VACUETTE® ESR BLOOD COLLECTION TUBES

Instruction for Use

Intended Use

VACUETTE® ESR tubes are used for the collection and transport of venous blood for blood sedimentation rate testing. ESR measurements refer to the Westergren method.

Product Description

VACUETTE® ESR tubes are plastic tubes with a pre-defined vacuum for exact draw volume. They are fitted with colour-coded VACUETTE® Safety Cap (13/75 mm tube) and Brom Butyl Caoutchouc Cap (9/120 mm tube). The tubes, additive concentrations, volume of liquid additives, and their permitted tolerances, as well as the blood-to-additive ratio are in accordance with the requirements and recommendations of the international standard ISO 6710 "Single-use containers for venous blood specimen collection".

The VACUETTE® ESR tubes contain a 3.2 % buffered tri-sodium citrate solution (0.109 mol/L). The mixing ratio is 1 part citrate solution to 4 parts blood. Tube interiors are sterile.

Storage

Store tubes at 4–25°C (40–77°F).

Note

Avoid exposure to direct sunlight. Exceeding the maximum recommended storage temperature may lead to impairment of the tube quality (i.e. vacuum loss, drying out of liquid additives, colouring, etc.)

Handling

Closed VACUETTE® **ESR System** (Fig. 5.4)

Equipment required for ESR measurements:

- A 9/120 mm, graduated, plastic tube with a citrate solution. Draw volume 1.5 ml and 2.75 ml.
- A9/120 mm glass tube with a citrate solution. Draw volumes of 1.6 ml or 2.9 ml are available.
- ESR rack with scale suitable for 1.5 ml/1.6 ml tubes, respectively ESR rack with scale suitable for 2.75 ml/2.9 ml tubes.

Procedures

After blood sampling and also before starting the ESR measurement, gently invert the tube 5–10 times to obtain the correct mixture. Use of a rotating mixer is recommended.

Note

It is recommended to do the determination within the first 4 hours when stored at room temperature. If longer storage is required, keep the specimen at the refrigerator (maximum 24 hours). Note that the sample must be brought to room temperature before use.

1. Place 1.5 ml, 1.6 ml or 2.75 ml, 2.9 ml tube into the corresponding rack vertically. Align the 0 mark at top of scale with the bottom of the meniscus of the blood at the blood-air interface.

   For the 1.5 ml/1.6 ml VACUETTE® ESR tube set timer for 30 minutes. The ESR

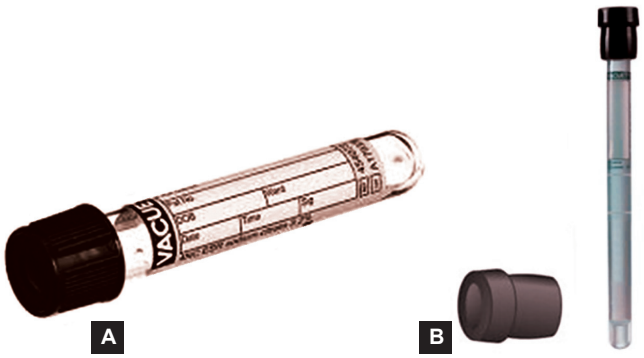

Fig. 5.4A and B: VACUETTE® ESR system

rack suitable for 1.5 ml/1.6 ml tubes delivers only the 1 hour Westergren value after 30 minutes reading time.

For the 2.75 ml or 2.9 ml ESR tube set timer for 60 minutes. The ESR rack for 2.9 ml tubes delivers the 1 hour and if required 2 hour Westergren value after 120 minutes reading time.

2. Discard VACUETTE® ESR tubes without opening.

Note

The conversion scale becomes highly compressed above Westergren values of 100 mm and ESR readings above this level should be repeated using the classic Westergren method if precise values are required.

1.5 ml and 1.6 ml tubes can be used with the following VACUETTE® ESR instruments: SRT 10/II, SRS 20/II, SRS 100/II.

The instrumentation allows for 1hour Westergren results after 15 minutes or 30 minutes.

(For further information contact Greiner Bio-One or see "VACUETTE® Automated ESR Systems Brochure")

Open VACUETTE® ESR System

The system consists of 3 parts:

1. A 13/75 mm plastic tube with a citrate solution.
2. A graduated pipette with rubber adapter.
3. ESR rack without any scale.

Procedure

After blood sampling and also before starting the ESR measurement gently invert the tube 5–10 times to obtain the correct mixture. Use of a rotating mixer is recommended.

Note

It is recommended to do the determination within the first 4 hours when stored at room temperature. If longer storage is required, keep the specimen at the refrigerator (maximum 24 hours). Note that the sample must be brought to room temperature before use

1. Remove the cap of the tube.
2. Insert the pipette into the opened tube and the blood will fill automatically to the zero-line of the pipette.

Note

If there is a bubble in the column of the pipette, the determination is not valid!

1. Place tube and pipette into the suitable rack. Tube and pipette must be in a vertical position.
2. After 60 and if required 120 minutes, read level between settled erythrocytes and the supernatant plasma from pipette.
3. Afterwards dispose of the tube and pipette together in a suitable biohazard disposal container.

Disposal

1. The general hygiene guidelines and legal regulations for the proper disposal of infectious material should be considered and followed.
2. Disposable gloves prevent the risk of infection.
3. Contaminated or filled blood collection tubes must be disposed of in suitable biohazard disposal containers, which can then be autoclaved and incinerated afterwards.
4. Contaminated ESR pipette and VACUETTE® tubes must be disposed of together in suitable biohazard disposal containers for infectious material.

Disposal should take place in an appropriate incineration facility or through autoclaving (steam sterilisation).

Use of ESR

1. **Diagnosis**
   a. **Marked elevation:** Multiple myeloma, macroglobulinaemia, tuberculosis, hyperfibrinogenemia, myocardial infarction, temporal arthritis, rheumatoid arthritis, chronic kidney disease, SLE, inflammatory bowel disease, polymyalgia rheumatica.

b. **Moderate evaluation:** Chronic infection (chronic osteomyelitis, chronic lung abscess, chronic bronchiectasis), rheumatoid arthritis, neoplasms (Hodgkin lymphoma, carcinomatosis, leukaemia), infective endocarditis, physiological (pregnancy), drugs (oral contraceptives, methyldopa, dextran, vitamin A, theophylline).

2. **Disease severity assessment:** ESR is a component of PCDAI (Paediatric Crohn's Disease Activity Index), an index for assessment of severity of inflammatory bowel disease in children.
3. **Monitoring response to therapy:** ESR has limited role to monitor the response to therapy in certain inflammatory disease such as rheumatoid arthritis, polymyalgia rheumatica and temporal arthritis. In Hodgkin lymphoma, ESR can be used as a crude measure to response. Also, it is used to define one of the several possible adverse prognostic factors in staging of Hodgkin lymphoma.

Causes of Slow or Decreased ESR

- Polycythaemia vera
- Sickle cell anaemia, spherocytosis, poikilocytosis
- Congestive heart failure
- Stages of severe dehydration like cholera, acute gastroenteritis
- Infections: Typhoid and undulant fever, trichinosis, malarial paroxysm, pertusis.
- Allergic states
- Drugs: Aspirin, cortisone, quinine.

In case of sickle cell anaemia, spherocytosis or poikilocytosis, there are abnormal red cells. These abnormalities of RBCs prevent rouleaux formation. Hence, decreased ESR.

Sources of Error and other Interfering Factors

- If the concentration of anticoagulant is higher than recommend the ESR may be elevated.
- Heparin alters the membrane zeta potential of RBCs and cannot be used as an anticoagulant.
- Tilting the pipette accelerates the ESR. The RBCs aggregate along the lower side, whereas the plasma rises along the upper side. Subsequently, the retarding of influence of the rising plasma becomes less effective. An angle of 3° from vertical position, may accelerate the ESR by as much as 30%.
- Plasma factors: An accelerated ESR is seen in elevated levels of fibrinogen and to a lesser extent of globulins. Albumin retards ESR. High rise of plasma viscosity also retards ESR. Cholesterol increases and lecithin decreases ESR.
- The test should be done within two hours. If the blood is stored for more than two hours, ESR will increase.
- If blood is kept in refrigerator, ESR is highly increased. So, refrigerated blood should be allowed to return to normal room temperature before the test started.
- Temperature of the environment: The ideal temperature for the test is 20–25°C. Increase in temperature is directly proportional to increased ESR.
- Bubbles left in the pipette, when the blood is filled, will affect ESR. The cleanliness of pipette is also important.
- Haemolysis may modify ESR.

Different Automated Methods of ESR

(Fig. 5.5A and B)

- Ves-Matic
- ESR STAT-PLUS
- SEDIMAT
- Zeta sedimentation

Advantages of Automated Methods

- Provide more rapid results
- Use small sample volumes
- Save technician time
- Provide increased safety because the need for sample manipulation is decreased.

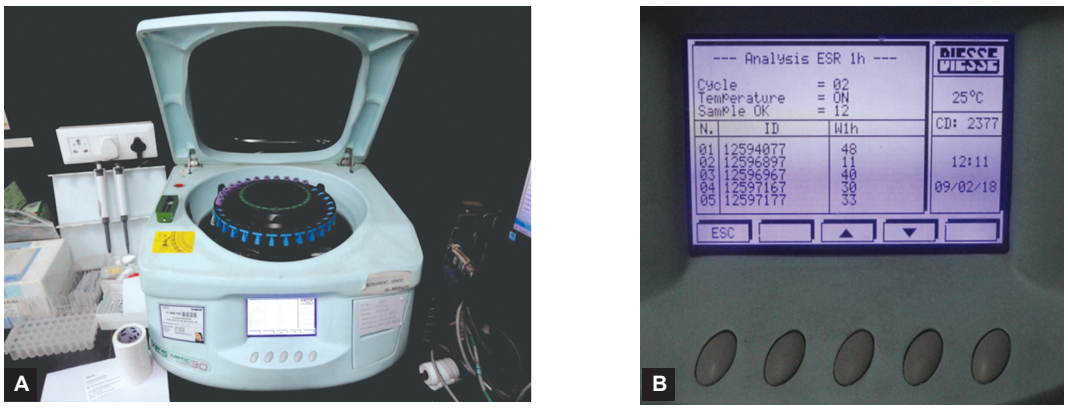

Fig. 5.5A and B: (A) Automated ESR machine; (B) Recording of ESR result

Zeta Sedimentation Rate (ZSR)

EDTA mixed blood (0.2 ml) is filled in a special capillary tube and is centrifuged in special apparatus (zeta fuge, Coulter electronics) for four times, each for 45 seconds. The capillary tube is mechanically rotated at 180° and centrifugation is done in reverse direction at every 45 seconds for four times. The red cell rouleaux develops better and travel down the capillary tube by alternate compaction and dispersion.

Result of ZSR: ZSR is expressed in terms of percentage.

Normal range in adults 40–50%. Rise in ZSR indicates rise in ESR.

Zeta crit: It is the ratio of the height of red cells to the total height of blood column.

Advantages of ZSR

- Requires small amount of blood (0.2 ml)
- No dilution is required.
- Eliminates the effect of anaemia.
- It is more sensitive than Westergren's method of ESR estimation.
- It requires minimum time.

Micro-ESR Method

Barrett (1980) described this micro-ESR method using 0.2 ml of blood to fill a plastic disposable tube 230 mm long with 1 mm inner diameter or internal bore. Both venous and capillary blood are suitable for this method. The tube filled with blood is kept vertically on a stand and the result is read after one hour.

This method has more utility in paediatric patients.

WINTROBE'S HAEMATOCRIT (PACKED CELL VOLUME)

The term 'haematocrit' theoretically means blood separation. Wintrobe haematocrit tube is mainly used for measurement of packed cell volume (PCV).

Definition of PCV

It is defined as the volume of packed red blood cells in a given sample of blood which is expressed as a percentage of the total volume of the blood sample.

Two methods are employed for measurement of PCV.

1. Macro-method using Wintrobe tube.
2. Micro-method using capillary tube.

1. Macro-method—Wintrobe's Tube

(Fig. 5.6)

It is a spherical, thick-walled glass tube 11 cm long and has an internal diameter of 2.5 mm with flat inner base. The tube is calibrated at 1 to 100 mm intervals and holds about 1 ml of blood. The markings on the tube are in

Fig. 5.6: Wintrobe's tube

reversed directions. Ascending marking is used for determination of PCV and descending marking is used for determination of ESR.

Uses

1. Wintrobe's tube is primarily used for determination of packed red cell volume (PCV) of blood.
2. Also it can be used for determination of ESR especially for anaemia correction with the help of correction curve.
3. Buffy coat smear preparation for demonstration of LE cell and staining with Leishman stain in diagnosis of SLE.
4. Abnormal or blast cells in aleukaemic leukaemia.

Blood and anticoagulant: Venous blood anticoagulated with double oxalate powder, EDTA powder or heparin.

Method of PCV Determination (Wintrobe Tube)

1. 2 ml venous blood is taken and immediately mixed with anticoagulant. Mix well by shaking.
2. The Wintrobe tube is filled with anticoagulated blood with the help of a long Pasteur pipette from the bottom up to mark '0' or '10' above.
3. The tube is then centrifuged at 3000 r.p.m. for 30 minutes.
4. The packed cell volume (PCV) is measured by noting the upper level of column of packed red cells by the markings in ascending order. PCV is expressed as percentage of the total volume of blood.

Zones Separated after Centrifugation

1. The layer of packed red cells or PCV is lower most which is usually 45 to 50%.
2. An intermediate thin layer comprises WBCs and platelets. It is above the lower most layer (red cells). The grey-coloured layer is known as buffy coat. Normally, this layer is 2 to 3%. Buffy coat layer is increased in leukaemia and severe degree of leukocytosis.
3. Upper most layer of plasma: This straw-coloured layer is above buffy coat layer and composed of free plasma. This layer may be pink in haemolysis, yellow in jaundice, and colourless in iron deficiency anaemia.

Normal Range of PCV

- Men: 45 ± 5%, i.e. 40 to 50%
- Women: 41± 5%, i.e. 36 to 46%
- At birth: 44 to 62%
- One year infant: 35% (approximate)
- 10 years: 37.5% (approximate)

Increased PCV: Polycythaemia, severe degree of dehydration, cholera, acute gastroenteritis.

Decreased PCV: Anaemia (usually less than 30%)

Sources of Error of PCV

- Inadequate duration and speed of centrifugation.
- Inadequate mixing of blood.
- Excess anticoagulant.
- Irregularity of the bore of Wintrobe tube.
- Trapping of leukocyte—platelet clumps in the tube will result defective red cell packing.

2. Micro-method—Capillary Tube Method for PCV

Nongraduated capillary tube (75 mm in length and about 1 mm internal diameter) is

rinsed with heparin solution (1:2000 heparin or 1 in 1000 dilution). Well dry this heparinised capillary tube at 56°C and stored.

When the test is done a capillary tube (haematocrit) is filled up with blood (finger prick or EDTA mixed venous blood) and the empty end is sealed with a micro-burner. The tube is filled up ½ to 2/3 of its length (not the entire length). The tube is then fitted on the micro-haematocrit centrifuge and centrifuged at 12000 r.p.m. for 5 minutes with the sealed end away from the centre.

Calculation of result: Then the capillary tube is taken out and PCB is calculated with the help of a millimetre rule commercially available.

Normal range of PCV (Table 5.1)

- Male: 47 ± 7%
- Female: 43 ± 5%

Advantages of Micro-method

- Time requirement for centrifugation is short.
- Small amount of blood is required to fill the capillary tube.
- Cost effective and easy to work.

Generally, PCV% is three times that of Hb g/dl or Hb%. So, if a person has haemoglobin of 15 g/dl, his PCV will be 15 × 3 = 45% (approx).

Table 5.1: Normal levels of packed cell volume (PVC) and haemoglobin

Age and sex	Packed cell volume (PCV)%	Haemoglobin (g/dl)
• Adult male	40–50	13–17
• Adult female (nonpregnant)	38–45	12–15
• Adult female (pregnant)	36–42	11–14
• Children, 6–12 years	37–46	11.5–15.5
• Children, 6 months to 6 years	36–42	11–14
• Infants, 2–6 months	32–42	9.5–14
• Newborns	44–60	13.6–19.6

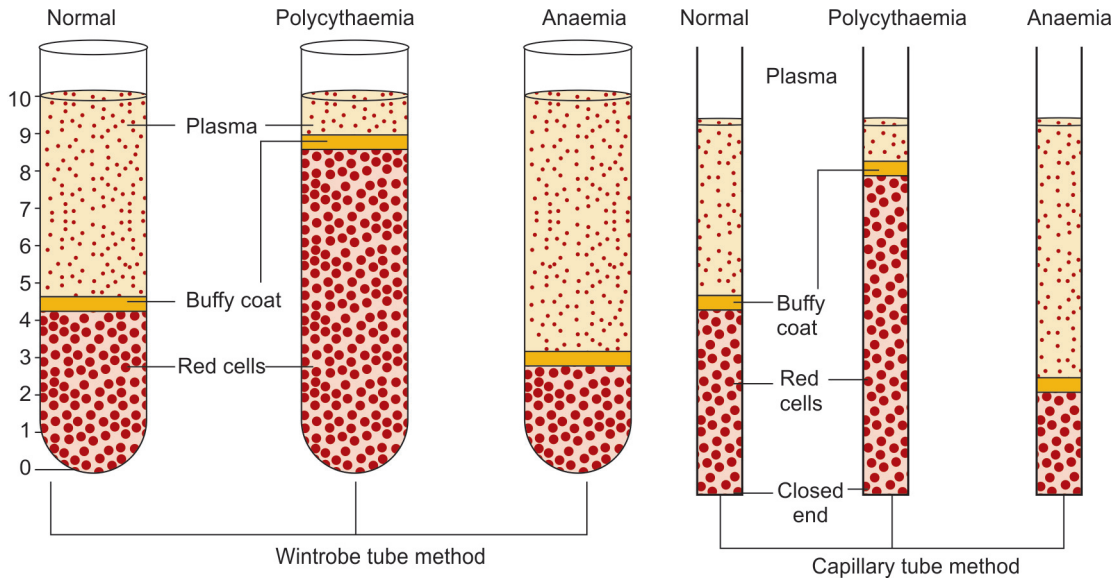

Fig. 5.7: Packed cell volume (PCV) shows comparison of normal, polycythaemia and anaemic blood samples by two different methods (Wintrobe and capillary tube)

VIVA VOCE

Q1. Why ESR is raised in anaemia?

Ans: i. In anaemia there is low erythrocyte mass compared to plasma. This change in the ratio of erythrocyte to plasma favours rouleaux formation and quicker sedimentation.

ii. Microcytes sediment more slowly and macrocytes somewhat more quickly compared to normocytes. The sedimentation of RBCs is directly proportional of RBC aggregates and inversely proportional to the RBC surface area. The microcytes have lower surface area to volume ratio.

Q2. Why ESR is low in sickle cell anaemia and spherocytosis?

Ans: Red cells with abnormal or irregular shape (poikilocytosis) hamper rouleaux formation. In sickle cell anaemia, the RBCs are abnormal in shape (sickle or crescentic in shape). So, ESR becomes low because of slower rouleaux formation.

In spherocytosis, because of the spherical shape (normal biconcave shape) RBCs have more surface area. As ESR is inversely proportional to the RBC surface, this increase in surface area causes decreased ESR.

Q3. How ESR can be used to monitor prognosis of disease?

Ans: ESR can be used to see the response to treatment in some diseases like tuberculosis, rheumatoid arthritis, polymyalgia rheumatica and temporal arteritis. If these diseases respond to treatment, the ESR tends to be lower over time.

In Hodgkin's disease, ESR, of less than 10 mm in first hour indicates good prognosis while ESR of more than 60 mm in first hour indicates poor prognosis.

Q4. Compare Westergren and Wintrobe methods of ESR as far as advantages and disadvantages are concerned.

Ans: Westergren method is more sensitive when ESR is high. Because ESR in this method has three phases with a longer second phase, so sinking of RBCs occurs better in a larger tube add longer second phase gives more accurate result when ESR is high.

But in Wintrobe's tube sinking of RBCs occurs quickly and packing is fast because it has a shorter tube length. So, Wintrobe's method is more sensitive when ESR is low.

Q5. Why Westergren's method is preferred to Wintrobe's method while estimating ESR?

Ans: i. When ESR becomes high Westergren method gives more accurate result.

ii. It is more sensitive because the pipette is longer and there are more markings (graduations).

Q6. What are advantages of Wintrobe's method?

Ans: In this method, ESR is estimated first and then the Wintrobe tube is centrifuged to get PCV. Moreover, the colour of plasma gives clues to certain diseases. Yellow plasma indicates jaundice, red-coloured plasma indicates haemoglobinaemia (intravascular haemolysis) and white in hyperlipidaemia (chyle).

Q7. What is automated ESR method?

Ans: The blood was drawn into special MONOSED vacutainers of Monitor 100® (1.6 ml, 120 mm long, 6 mm diameter) with 1.28 ml of automatic draw containing 0.32 ml of 3.2% sodium citrate. The blood citrate mix reaches up to a maximum length of 60 mm from the bottom of the

tube. After proper mixing, the samples were immediately transferred to the analyzer. The ESR reading is taken through a 45 mm high window, 2 mm above the maximum sample level. The Monitor 100® has the advantage of giving the result of 100 samples in 30 minutes (equivalent to 1 hour Westergren reading) and 60 minutes (equivalent to 2 hours Westergren reading). The machine Monitor 100® supplied by Electra Lab, Italy.

Marked discrepancy in the ESR result was noted for high ESR values when compared between manual and automated methods. But it was not seen for normal ESR values. So, a correction factor to be applied when ESR is very high for this automated method.

Q8. What are the length and diameter of Wintrobe's tube? What amount of blood it can hold?

Ans: The tube has length of 110 mm or 11 cm and internal diameter of 3 mm. It is graduated at 1 mm intervals and marked 0 to 100 mm (10 cm) from above downward and also from below upwards.

The tube can hold about 1 ml of blood.

Q9. How PCV is used to determine red cell indices?

Ans: i. Mean corpuscular volume (MCV)

It is the average volume of RBC and is calculated from red cell count and haematocrit volume

MCV = PCV in L/L ÷ RBC count/L (normal value is either 85 ± 8 fl or 77–93 fl)

ii. Mean corpuscular haemoglobin (MCH)

It is the content by weight of haemoglobin of average red cell.

MCH = Hb/L ÷ RBC count/L (normal range is either 29.5 ± 2.5 pg or 27–32 pg)

iii. Mean corpuscular haemoglobin concentration (MCHC)

It is the average of haemoglobin concentration and haematocrit value which is expressed in terms of PCV (0.45 deciliter normally).

MCHC = Hb/dl ÷ PCV in L/L (normal range is either 32.5 ± 2.5 g/dl or 30–35 g/dl).

As MCHC is independent of RBC count and size, it is considered to have greater clinical significance as compared to other red cell indices. It is low in iron deficiency anaemia but usually normal in macrocytic anaemia.

Clinical significance of red cell indices

- In iron deficiency anaemia and thalassaemia, MCV, MCH and MCHC are reduced.
- In anaemia due to acute blood loss and haemolytic anaemias, MCV and MCH are usually within normal limits.
- In megaloblastic anaemia, MCV and MCH are high but MCHC is usually normal. This is because the amount of haemoglobin increases proportionately with the increase in cell size. Hence, MCHC remains normal though MCV and MCH are high.

Q10. What are the values of red cell indices (absolute values) when there is both iron and folate deficiencies?

Ans: Anaemia is macrocytic and hypochromic. So, MCV is high, MCH is low or normal and MCHC is low.