



National Policy and Procedure for manual erythrocyte sedimentation rate (ESR) test (Westergren and Modified Westergren method)

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Acronyms:

ESR	Erythrocyte Sedimentation Rate
EDTA	Ethylenediaminetetraacetic acid
PPE	Personal Protective Equipment
QC	Quality Control
CV	Coefficient of Variation
LIS	Laboratory Information System (Al Shifa system)
MOH	Ministry of Health
Mm/hr	Millimeter per 1 hour
RBC	Red blood cells
SOP	Standard operating procedure

1. Purpose

This document describes the standardized procedure for performing the Erythrocyte Sedimentation Rate (ESR) test manually using the Westergren method to ensure accurate, reliable, and reproducible results for patient diagnosis and monitoring.

2. Scope

This document is applicable to all medical laboratories under MOH.

3. Definitions:

3.1. ESR (mm/hour): is the rate at which erythrocytes settle in anticoagulated blood under standardized conditions over one hour.

3.2. Westergren tube is a long, vertical, graduated glass or plastic tube with a 200 mm graduated scale and 2.5 mm internal diameter used to measure the erythrocyte sedimentation rate (ESR) .

4. Policy

4.1 The laboratory must ensure that all personnel performing the test are adequately trained and deemed competent.

4.2 Only properly collected EDTA sample shall be accepted for analysis. Specimens must be processed within the defined stability period and handled under appropriate conditions to avoid pre-analytical errors.

4.3 Quality control procedures shall be implemented and documented regularly to ensure accuracy and reliability of results. All equipment, including Westergren tubes and racks, must be properly maintained, calibrated, and verified according to laboratory quality standards.

4.4 ESR results shall be reviewed, validated, and reported by authorized laboratory personnel. Any abnormal findings must be communicated according to the laboratory's reporting policy. All procedures must comply with institutional policies, safety regulations, and applicable accreditation requirements.

5. Procedure

5.1. Clinical background:

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is used for suspected inflammatory conditions. Although a rapid sedimentation rate is indicative of tissue destruction (whether degenerative or inflammatory) and is found in most bacterial

infections, the ESR is not considered a very reliable screening method as it can be elevated when there is no disease and can be normal when disease is present.

It also does not indicate the type of disease but finds its greatest use in following the progress of a particular illness rather than in its diagnosis.

There are, however, several groups of patients where the ESR is important, such as patients suspected of having temporal arteritis or polymyalgia rheumatica, or those with rheumatoid arthritis, tuberculosis, or Hodgkin's disease.

ESR is reported in mm per hour which is the sedimentation of red cells in diluted blood after standing for 1 hour in an open-ended glass tube of tube with a 200 mm graduated scale and 2.5 mm internal diameter mounted vertically on a stand. (see figure 1)

5.2. Principle:

Erythrocytes suspended in plasma settle under gravity. Aggregation (rouleaux) accelerates sedimentation. In the Westergren method, diluted whole blood is placed in a standardized vertical tube (200 mm scale, 2.5 mm internal diameter). After 60 minutes, the fall of RBC column is read as mm/hour. (see figure 1)

5.3. Patient preparation

No special preparation

5.4. Sample requirements

5.4.1 The Westergren ESR method requires blood anticoagulated in a 4:1 blood-to-anticoagulant ratio using 3.8% (or 3.2%) sodium citrate as the anticoagulant. This may be achieved through one of two approaches depending on the system available at each laboratory:

Option A (modified Westergren ESR) — Prefilled citrate ESR tube systems:

Collect whole blood into a standard EDTA tube. The ESR system tube (e.g., Vacuette ESR, Greiner; Starstedt ESR; or equivalent) is prefilled by the manufacturer with the correct volume of sodium citrate. Insert the EDTA blood into the ESR tube according to the manufacturer's instructions to achieve the 4:1 ratio automatically. Follow manufacturer's instructions for mixing. (see figure:2)

Option B — Manual citrate dilution method: Collect whole blood into a standard EDTA tube. Add 3.8% sodium citrate manually to the blood sample at a 4:1 ratio

(4 parts blood: 1 part citrate). Mix gently by inversion (8–10 times) before transferring to the Westergren tube or pipette.

5.4.2 Perform test within 4 hours of collection at room temperature (18–25°C). If delayed, store at 2–8°C and test within 24 hours. Sample must be brought to room temperature before testing (at least 30–60 minutes before testing).

5.5. Specimen rejection criteria:

- Incorrect labeling
- Clotted sample
- Insufficient quantity.
- Hemolyzed sample
- Inappropriate container.
- Inappropriate transport /storage
- Unknown duration of delay.

5.6. Equipment and Materials:

- Westergren ESR stands with calibrated tubes (200 mm)
- ESR pipettes or disposable Westergren tubes
- ESR racks (vertical alignment)
- Timer or stopwatch
- Personal protective equipment (PPE)
- Cleaning solution and 70% ethanol
- Distilled water for cleaning

5.7. Safety precaution:

Proper protective clothing (gloves and laboratory coat) must be always worn when handling potentially infectious material (patient samples).

5.8. Procedure Details:

5.8.1 Mix EDTA tube gently (8–10 inversions) to ensure uniform suspension (avoid frothing).

5.8.2 Make sure that the ESR tube is prefilled with sodium citrate otherwise you must add sodium citrate to the sample manually at ratio of 4:1.

5.8.3 Insert the disposable plastic Westergren tube gently into the sample ensuring the tube reaches the bottom.

- 5.8.4 Adjust the column of the blood to the zero mark on the tube by raising the tube from the bottom of the cap without bubbles.
- 5.8.5 The tube should be held vertically, protected from direct sunlight, draughts and vibration and kept at a constant temperature (± 1 C) within the range 18–25 C during the sedimentation period.
- 5.8.6 Start the timer immediately after placing the tube.
- 5.8.7 After 60 minutes, record the distance (in mm) between the plasma meniscus and the top of the red cell column.
- 5.8.8 Record results as mm/hour.
- 5.8.9 Dispose of the used tube as per biohazard waste protocol.

5.9. Quality control:

5.9.1. Internal quality control

- Use commercially available ESR control materials with assigned target values and acceptable ranges (normal and abnormal levels). Controls must be validated upon receipt and each new lot number before use in patient testing.
- Run at least one normal and one abnormal control each working day before reporting patient results.
- **Acceptability criteria:** Results are acceptable when control values fall within ± 2 SD of the established mean, or within the manufacturer's stated acceptable range, whichever is more stringent. A coefficient of variation (CV) of $\leq 10\%$ is the recommended performance target for ESR.
- **Rejection criteria and corrective action:** If a control result falls outside the acceptable range:
 1. Do not release patient results.
 2. Repeat the control using a freshly prepared sample
 3. Check for pre-analytical variables: tube verticality, room temperature (18–25°C), timer accuracy, and correct anticoagulant ratio
 4. If the repeat also fails, remove the batch from use, investigate the cause, document findings, and notify the laboratory supervisor
 5. Patient results may only be released after the problem is resolved and the control is back in range

- Ensure vertical alignment of tubes and stable room temperature (18–25°C).
- Check timer accuracy monthly using a calibrated reference timer
- Calibrate ESR stands annually or as required.
- Document and investigate QC outliers.

5.9.2. External quality control: Recommended

5.10. Reference interval

Normal Values:

Adult:

- Males: 0-15 mm
- Females: 0-20 mm

Children

- Neonates (0–14 days): 0-2 mm/hr.
- Infants and children (>14 days to puberty): <10 mm/hr

Elderly:

A single fixed reference interval is not appropriate for elderly patients. The upper limit of normal ESR can be calculated using the Miller formula: for men, age in years divided by 2; for women, (age in years + 10) divided by 2. For patients of the same age, the ESR by the Miller formula should be 5 mm/hr higher in women than men.

Examples for clinical guidance:

Age	Male upper limit (age/2)	Female upper limit ((age+10)/2)
50 years	25 mm/hr	30 mm/hr
60 years	30 mm/hr	35 mm/hr
70 years	35 mm/hr	40 mm/hr
80 years	40 mm/hr	45 mm/hr

Pregnant:

- Early gestation: up to 48 mm (62 if anemic)
- Late gestation: up to 70mm (95 if anemic)

Note: ESR values in pregnancy should be interpreted in the context of gestational age and hemoglobin concentration. Laboratories are encouraged to establish local reference intervals where feasible.

5.11. Recording the results

Record the results in LIS (Al Shifa) as mm in hour

5.12. Limitation of the test

5.12.1. Effect of plasma proteins:

Changes in plasma proteins occur rapidly following tissue injury or in response to inflammation. Increased concentration of fibrinogen and immune globulins will increase rouleaux formation and hence the rate of sedimentation. Plasma albumin retards sedimentation of RBCs.

5.12.2. The RBC count and number:

The size and the number of RBCs also affect the ESR, the cells which show alteration in the size like spherocytes and sickle cells, usually do not exhibit increase rate, unless there is severe anemia. Increased red cells mass, retards the sedimentation rate, e.g. polycythemia while anemia increase ESR due to reduce RBC mass

5.12.3. Technical factors

The tube should be vertical and there should be no any vibration which can reduce the ESR

Temperature (RT 18-25 C°): higher temperature can cause false high results due to the reduction in the plasma viscosity.

5.12.4. Pregnancy: Can lead to High ESR values.

5.12.5. Drugs: Steroids and immunosuppressant can decrease the ESR values while oral contraceptive can increase ESR values

5.12.6. Age: ESR varies with age and increases as age advances

6. Responsibilities

6.1 Laboratory Program committee:

- Ensure that document is approved by ministry of Health and distributed to all institutions.

6.2 Task force creating the document:

- Ensure that the document is in accordance with Ministry of Health guidelines and is evidence based.
- Ensure that the document is sent to the relevant individuals in different health institutions for review.
- Ensure that the document is modified after receiving relevant feedback from reviewers.
- Ensure that the document is updated where appropriate.

6.3 Laboratory Head/ supervisor:

- Review the document and provide feedback where appropriate.
- Ensure that local standard operating procedures are in line with document.
- Ensure that all staff are trained and adhere to the document

6.4 Technical staff

- Be familiar with this document and adhere to it where applicable

7. Document History and Version Control

Version	Description	Review Date
1	Initial Release	August 2031

8. References

- International Council for Standardization in Haematology (ICSH). Recommendations for measurement of erythrocyte sedimentation rate (ESR). Am J Clin Pathol. 2017.
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9. Annexes

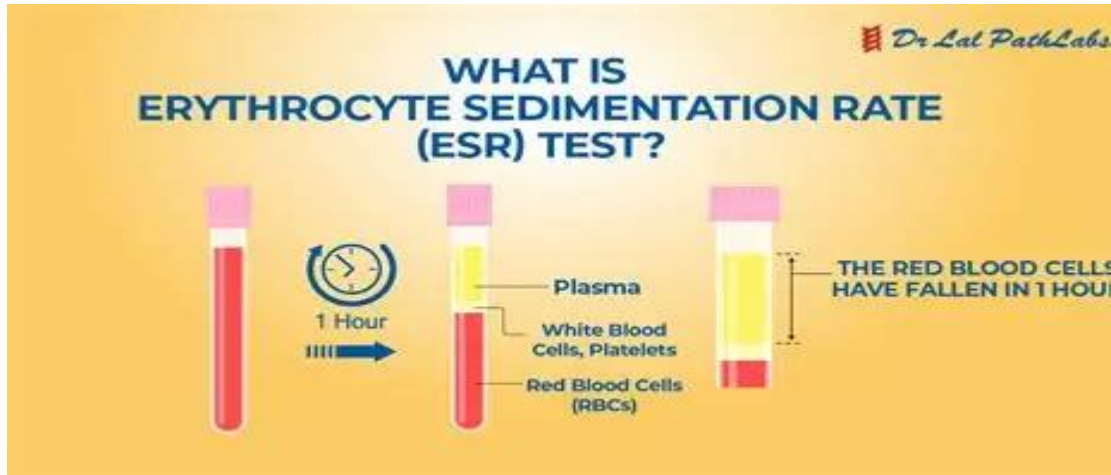


Figure 1: principle of ESR testing (adopted from www.lalpathlabs.com/blog/esr-test)



Figure 2: ESR test using prefilled tubes with sodium citrate anticoagulant