Basics of Erythrocyte Sedimentation Rate

Erythrocyte Sedimentation Rate

The Erythrocyte Sedimentation Rate (ESR) laboratory test is a nonspecific test that is performed to aid in evaluating varying degrees of inflammation. ESR results above 13 for males and above 20 for females exceed the normal upper limits and can be considered abnormal (Clinical and Laboratory Standards Institute [CLSI], 2011). An elevated ESR can be an indicator of acute inflammation or infection.

Origin of the ESR test

The ESR theory was first observed by Polish physician Dr. Edmund Faustyn Biernacki in 1897. Biernacki observed increased ESR values in patients that also had increased levels of fibrinogen, a plasma protein that promotes platelet aggregation. Following this discovery, in 1918, Swedish hematologist Dr. Robert Fahraeus studied the ESR values of pregnant and not pregnant women. Fahraeus found that pregnancy caused an elevation in ESR (Aytekin, 2018). A few years after Fahraeus' discovery, in 1921, Swedish internist Dr. Alf Vilhelm Albertsson Westergren studied the ESR results of patients with pulmonary tuberculosis. Westergren went on to establish blood sampling methods and standards for ESR testing that are still used today (Grzybowski & Sak, 2011).

Red blood cell aggregation and its relation to sedimentation

The ESR phenomenon can be described in three phases. When plotted on an ESR vs time graph (Figure 1), a sigmoid curve will typically result within 60 minutes. The three phases are referred to as (1) lag, (2) decantation and (3) packing. During phase 1 (lag phase), considered the most important phase of the sedimentation process, individual red blood cells (RBC) stick together to form aggregates that resemble stacks of coins which are 2D aggregates called Rouleaux. Shortly after the formation of the 2D aggregates, they get larger and form a 3D aggregates. Formation of 3D aggregates is complete within minutes. Sedimentation is slow in this phase due to the red cells weighing almost the same as the plasma surrounding them. Phase 2 is the decantation phase, and due to the imbalance of volume and surface area ratio of the Rouleaux, RBC aggregates start to fall more rapidly. In response to the RBC aggregates falling more rapidly, the plasma rises to the top. Phase 2 is followed by the packing phase where sedimentation occurs at a slower pace due to the RBC aggregates beginning to pile or "pack" together at the bottom of the tube. The extent of packing is determined by the hematocrit of the sample. The hematocrit is the volume of red cells in the total blood sample, expressed in a percentage.

The sedimentation rate of RBCs is determined by the difference in weight of RBCs vs. plasma. However, once the RBCs aggregate and form Rouleaux, the aggregates weigh more than the plasma and the sedimentation rate increases in phase 2. Therefore, ESR indicates the extent of RBC aggregation. (Baskurt, et al, 2019) As stated by TL Fabry, there can be no sedimentation if aggregation does not occur first. (Fabry, 1987)

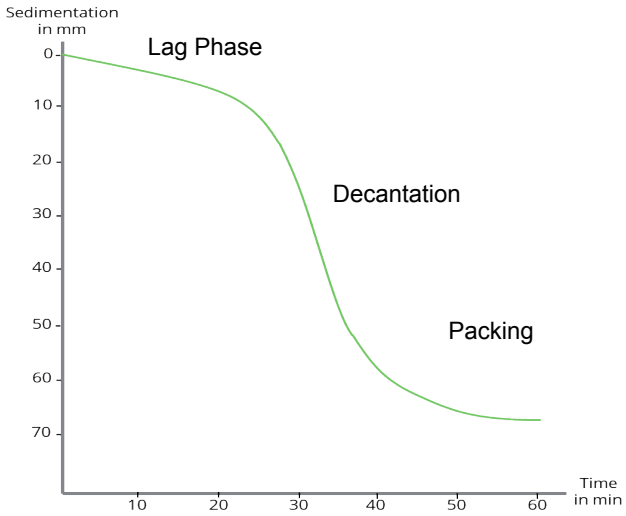

Figure 1: Sigmoid Sedimentation Curve

There are many variables that can affect ESR. Acute-phase proteins are released in cases of tissue damage, inflammation, infection, malignancy, pregnancy, etc. For example, when injury has occurred in the body, the plasma protein fibrinogen is increased. Since fibrinogen is a positive plasma protein that promotes clotting, Rouleaux formations develop faster during a state of injury or inflammation. Due to the increase in aggregation of erythrocytes and the consequential effects of gravity, ESR elevates. Another common biomarker for inflammation is C-reactive protein (CRP). This acute phase protein is increased during the first 24 hours after injury or inflammation then drops back to normal levels. In contrast, ESR can continue to elevate for many days after injury or inflammation due to the release of other acute-phase proteins, such as fibrinogen. Therefore, ESR is a helpful biomarker not only for diagnosis, but continued monitoring of the progression of the body's inflammatory state. (CLSI, 2011)

The standardized ESR test procedure (CLSI, 2011)

Blood collection

- In less than 30 seconds, obtain a non-hemolyzed blood specimen by venipuncture. Be sure to immediately mix the specimen thoroughly with EDTA anticoagulant. Blood specimens should be examined for clots that could invalidate the results.

Time of the test

- Ideally, the testing process should begin within four hours of the specimen collection (when held at room temperature). If a longer storage time is necessary, testing can begin up to 24 hours after specimen collection if the specimen is refrigerated and brought back to room temperature for 15 minutes before testing begins.

Specimen preparation

- For standard tubes, there should be a minimum of 12 complete inversions performed with the air bubble traveling back and forth, from the top to the bottom, of the tube. When using nonstandard tubes, the required number of inversions should be reassessed to ensure proper mixing as this is a critical step in obtaining accurate results. The mixing inversions should be performed immediately before the pipette is filled. Remember to let refrigerated specimens return to room temperature for at least 15 minutes before beginning the mixing inversions followed by pipetting.

Blood cell suspension

- Resuspend the anticoagulated specimen by diluting an aliquot of the EDTA specimen at a 4:1 proportion in sterile trisodium citrate dihydrate concentration of 100 to 136 mmol/L. Physiological saline can also be used as a diluent using the same 4:1 ratio.

Handling of the pipette

- Using a mechanical suction device, aspirate the diluted and bubble-free sample into a clean and dry Westergren pipette. Fill the Westergren pipette exactly to the 0 mark. Place the filled pipette in the vertical position, level and at 18-25 degrees Celsius. Make sure the area is free from vibrations, drafts, direct sunlight or any other factors that might impact the result.

Reading of the test

- After 60 minutes, read the distance, in millimeters, from the bottom of the plasma meniscus to the top of the column of sedimented erythrocytes. Do not include any leukocytes, or the buffy coat, in this reading. Record this value.

Reporting of the results

- Results are reported in millimeters and represent the distance between the meniscus of the plasma, at the top of the column, and the top of the erythrocyte cell column where the RBCs have settled in the column. Results are only valid if obtained at the 60-minute marker, with a one-minute variance. Record the result as mm/hour.

Material requirements for the ESR test (CLSI, 2011)

Westergren Supplies

Pipette

- The pipette tube should be colorless, circular, length of at least 200 mm.
- If the reading of the pipette is not done electronically, a sedimentation scale is marked on the pipette or next to it. It should have clearly marked lines in increments of 1mm to 200mm from the bottom of the pipette.
- Pipette diameter should not be less than 2.55 mm. Diameter should be within 5% throughout the length of the pipette. The interior of the pipette should be a cylinder with the difference between the long and short axes not exceeding 0.1 mm.
- The pipette can be disposable, glass or plastic. If plastic, the pipette should not have adhesive properties towards erythrocytes and should not release plasticizer that alters sedimentation.
- If reusable, pipettes must be washed in cold running water, disinfected for one hour, rinsed thoroughly in distilled water and dried in an incubator at 37 degrees Celsius for one hour.

Pipette rack

- During testing, the pipette must remain vertical and motionless.
- Only plumb bob racks have a leveling device that ensures that pipettes will remain vertical.
- The pipette rack must also ensure that no blood can leak from the pipettes.

The selected routine method

At present, the working method recommended by CLSI is the same as the standardized method. However, other procedures including those based on automated analyzers are acceptable as working methods. The standardized method is often used as a reference method when exploring automated methods of testing (CLSI, 2011).

Clinical interpretation of the ESR test

Conditions associated with ESR testing

- Rheumatoid arthritis, rheumatic carditis, temporal arteritis, vasculitis, systemic lupus erythematosus and other autoimmune conditions
- Multiple myeloma and other paraproteinemia conditions
- Hodgkin's lymphoma and other malignancies
- Inflammatory bowel conditions

ESR testing can be a useful biomarker that helps health care providers reach a diagnosis or evaluate a current condition state. The conditions listed above are common differential diagnoses where a provider might obtain an ESR value to evaluate the severity of the inflammation process (CLSI, 2011).

Reference Values

Mean ESR Reference Values for the Westergren Method (mm/hr)			Upper Limit of Normal	
Age (Years)	**Male**	**Female**	**Male**	**Female**
18-30	3.1	5.1	< 7.1	< 10.7
31-40	3.4	5.6	< 7.8	< 11.0
41-50	4.6	6.2	< 10.6	< 13.2
51-60	5.6	9.4	< 12.2	< 18.6
60-70	5.6	9.4	< 12.7	< 20.2
Over 70	5.6	10.1	< 30	< 35

(CLSI, 2011).

Physiological and clinical factors that increase the ESR

Conditions (see conditions associated with ESR) that cause increased levels of immunoglobulins, fibrinogen or other clotting factors.

Physiological and clinical factors that decrease the ESR

Conditions that cause delayed stacking of erythrocytes or abnormally shaped erythrocytes such as polycythemia and sickle cell anemia. Increases in plasma viscosity can also impede the speed of erythrocytes sedimentation.

Common errors in ESR testing (CLSI, 2011)

Specimen collection

- Different specimen collection methods can cause differences in comparability. The collection methods include collecting with EDTA or collecting in a special sedimentation rate evacuated tube that dilutes four volumes of blood with one volume of citrate solution.

Time and temperature control

- Testing should be started within four hours of collection when the specimen is held at room temperature. When the specimen is refrigerated, testing can be done up to 24 hours after the collection if it is returned to room temperature for 15 minutes before testing.
- During sedimentation, the tubes should be kept at a temperature of 18 to 25 degrees Celsius. Be sure to keep the tubes out of direct sunlight and away from heating or cooling vents.

Tube diameter

- When tubes are too narrow, an increase in variability will occur. If tubes are too narrow, the routine method will be lower than expected. Pipette diameter should not be less than 2.55 mm

Inadequate material

- Plastic pipettes may be used provided that the manufacturer demonstrates that they are not affected by mold-release agents during the manufacturing process. It must also be demonstrated that the plastic pipettes do not contain plasticizers that interact with blood. Some plastics strongly attract erythrocytes and can cause plugging of the erythrocytes.

Defective equipment

- Allowing evacuated tubes to fill completely can help to assure the degree of vacuum in the tube does not affect final dilution of the results.

Dilution errors

- Dilution is critical for preventing plugging of the erythrocytes. The dilution step should be diligently performed to prevent variability in testing results.

Vibration / Verticality

- Vibration can cause a positive bias in the routine method. Therefore, reproducibility is potentially affected. It is essential that the bench on which the testing is performed is stable.
- If the tubes or pipettes do not remain vertical, the ESR results can become extremely altered. False positives can occur if the verticality of the pipette or tube is even two degrees off.

Technical innovation and advancement to ESR testing

Since the introduction of the Westergren method, there have been several developments in sedimentation rate testing. These developments include reducing biohazard and testing time. With the use of automated and semiautomated systems, turnaround time is greatly reduced without compromising the validity of the test. Some instruments use infrared beam, laser beam and quantitative photometry to determine erythrocyte sedimentation rates. Verification of these innovations can be done by comparing the results to the Westergren reference ranges. Manufacturer's should provide data on the reliability and accuracy of their instrument and testing method, calibration, and control procedures (CLSI, 2011).

Conclusion

Over the past century, innovation in ESR testing has evolved the methodology to be safer, more reliable and faster. Although the Westergren method is still considered the gold standard for ESR testing, automated analyzers have improved this process and show high correlation results. When choosing a testing method, Clinical and Laboratory Standards should be referenced for more information regarding validation, verification, quality assurance, and quality control.

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