EXERCISE 9: ERYTHROCYTE SEDIMENTATION RATE (ESR)

Skills 20 points

Objectives

1. State the principle of the Erythrocyte Sedimentation Rate (ESR) test.
2. List four (4) patient factors that affect the ESR test.
3. Define rouleaux and how it affects the ESR.
4. Describe the relative amount of RBC settling seen with a higher ESR and a lower ESR.
5. Describe three (3) stages that occur during the sedimentation of the red cells.
6. State the clinical significance of the Erythrocyte Sedimentation Rate.
7. List five reasons why erythrocyte sedimentation rate (ESR) tests are performed.
8. List two types of blood samples that can be used for the ESR.
9. State the sample type and advantage one sample has over the other.
10. State the length of time blood samples may be used for the ESR test based on storage conditions.
11. Identify five (5) mechanical and technical factors that can affect the ESR.
12. Using proper units, state the normal values for the ESR for men and women.
13. List nine sources of error that would adversely affect the results of the ESR test.
14. Abiding by safety protocol specific to performing the ESR, correctly perform at least one (1) Westergren sedimentation rate test and read an additional two (2) others.

Discussion

The erythrocyte sedimentation rate (ESR), sometimes called the Sed Rate, is a simple inexpensive laboratory test performed in the hematology department. Sedimentation is the term used to describe the process of solid particles settling to the bottom of a liquid.

The principle of the ESR can be summarized as follows: *A sample of well-mixed whole blood is carefully diluted and placed in a slender tube, which is then placed in a stationary, vertical position. The distance the red cells (erythrocytes) fall, or settle, in a specific amount of time is the Erythrocyte Sedimentation Rate (ESR).*

The ESR is directly proportional to the RBC mass and inversely proportional to plasma viscosity. Patient physiological factors that affect the ESR include the following:

1. RBC size
2. RBC shape
3. Plasma fibrinogen
4. Plasma viscosity

In normal whole blood, the RBC mass is small and therefore the ESR is decreased (cells settle out slowly). In abnormal conditions when RBCs can form rouleaux, the RBC mass is greater, thus increasing the ESR (cells settle out faster). The term *rouleaux* refers to the alignment of the RBCs into a stack, similar in appearance to a stack of coins, caused by extra or abnormal proteins in the blood that decrease the normal distance RBCs maintain between each other.

Any condition that allows a faster fall of the RBCs, the further they will settle, and the higher the ESR. Conversely, the slower that RBCs fall, the less they settle, and the lower the ESR.

The sedimentation of RBCs takes place in three stages:

- **First Stage** – (first 10 minutes of test) ‘Rouleaux’ formation, the sedimentation rate is slight
- **Second Stage** – (next 40 minutes of test) Sedimentation occurs at a fairly rapid rate. Most of the settling takes place during this time.
- **Third Stage** – (final 10 minutes of test) Sedimentation rate is slow because of the accumulation of RBCs in the bottom of the tube. The packing of the cells occurs.
Clinical Significance
The ESR is a **nonspecific** indicator of inflammation and necrosis. Since it is not a specific test, the ESR is performed for the following reasons:

1. To screen for certain disease conditions
2. To differentiate among diseases with similar symptoms
3. To serve as an index to disease severity
4. To monitor the course of an existing disease
5. To monitor a patient’s response to treatment

A rising ESR can mean an increase in inflammation or a poor response to a therapy; a decreasing ESR can mean a decrease in inflammation or a good response to therapy.

<table>
<thead>
<tr>
<th>Increases Sed Rate</th>
<th>Decreases Sed Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rouleaux formation</td>
<td>Microcytes</td>
</tr>
<tr>
<td>Macrocyes</td>
<td>Poikilocyes</td>
</tr>
<tr>
<td>Elevated Fibrinogen</td>
<td>Sickle Cells</td>
</tr>
<tr>
<td>Excess plasma immunoglobulins (ex. Multiple Myeloma)</td>
<td>Spherocytes</td>
</tr>
</tbody>
</table>

The Westergren method is preferred by NCCLS standards because of its simplicity and greater distance of sedimentation measured in the longer Westergren tube. The straight tube is 30 cm long and calibrated in millimeters from 0-200. Approximately 1 mL of blood is required.

Specimen Acceptability and Storage
A fresh whole blood sample collected in either EDTA (purple-stoppered tube) OR buffered sodium citrate (black-stopper tube) appropriately filled is acceptable for testing. All specimens must be checked for the presence of clots. If clots are found, the specimen must be recollected.

The purple top tube must be properly diluted as part of the ESR test procedure, but can be used for other hematology test. The advantage of the black stopper tube is that the test can be performed directly from the tube. The black stopper tube may not be used for any other test.

Room temperature stored specimens must be assayed within 2 hours of collection. Refrigerated specimens can be stored up to 24 hours before assayed. Refrigerated specimens must be brought to room temperature and well mixed before testing can begin.

Testing Considerations
Mechanical and technical factors that are associated with the testing of the specimen can also affect the test results. As with any laboratory procedure, a documented protocol must be followed. The following mechanical and technical factors must be kept in mind:

1. Air bubbles in the tube will interfere with accuracy. Careful pipetting and diluting are critical.
2. Tubes must be kept exactly vertical during testing.
3. Test area must be free of vibrations. Avoid areas close to centrifuges or other equipment.
4. The test sample and the room temperature must both be at 20-25°C (68-77°F).
5. The test must be read promptly at one hour for accurate results.
6. Quality control and Quality assurance must be performed and documented.
Expected Values - Normal range/Reference Values (Westergren method)

- Males under 50 years: 0-15 mm/hr
- Males over 50 years: 0-20 mm/hr
- Females under 50 years: 0-20 mm/hr
- Females over 50 years: 0-30 mm/hr
- Newborns: 0-2 mm/hr
- Neonates to puberty: 3-13 mm/hr

C-Reactive Protein

Another laboratory test called the C-Reactive Protein (CRP) test is a much better indicator of inflammation and necrosis than the ESR. CRP is the actual protein being produced. The CRP determines the amount of protein present and is not affected by anemia or abnormal serum proteins. However, the ESR remains a very popular test to indicate the presence of inflammation and necrosis, and is relatively inexpensive. ESR and C-reactive protein (CRP) are both markers of inflammation. Generally, ESR does not change as rapidly as does CRP, either at the start of inflammation or as it goes away.

Sources of Error

1. Concentration of the anticoagulant is incorrect. If the specimen is collected in the black stopper tube (buffered sodium citrate) and is under filled, the excess anticoagulant and diluent will result in a decreased ESR. (If EDTA samples are used for the ESR, the sample is manually diluted prior to performing the test.)
2. Not reading the ESR test at the appropriate time. Allowing the ESR to stand for more than 60 minutes, the results will be falsely elevated. If the test is read before the 60 minutes time, falsely low values are obtained.
3. An increase or decrease in room temperature may lead to increased or decreased ESR results. The temperature should be within the range of 20-25 C.
4. Not maintaining the tubes in an upright position during the test. All sedimentation racks used should be equipped with leveling screws and a spirit bubble. Tilting of the ESR tube increases the sedimentation rate.
5. Bubbles created in the blood tube when filling will lead to a decreased ESR.
6. Fibrin clots - the presence of fibrin clots in the blood sample invalidate the test results.
7. Hemolysis of the specimen causes a decrease in the ESR.
8. Not setting up the test within the appropriate time. It is important that samples stored at room temperature are set up within two hours of blood collection. Refrigerated (4 C) specimens can be stored up to 24 hours before tested.
9. Not allowing refrigerated specimens to return to room temperature before testing.
10. Not mixing the blood sample before testing. All samples must be well mixed before testing.

References

1. The Erythrocyte Sedimentation Rate: Old and New Clinical Applications, date unknown, Constantine, Saadeh, MD
   http://www.mechatronics.nl/technics/esr/the_erythrocyte_sedimentation_rate_by_c_saadeh_md.pdf
EXERCISE 9: ERYTHROCYTE SEDIMENTATION RATE (ESR)

Specimen
Fresh whole blood sample collected in EDTA (purple-top tube). Specimen must be checked for the presence of clots. If clots are found, the tube must be recollected.

Equipment (Modified Westergren Method)
1. Well mixed EDTA blood.
2. Blue reservoir, prefilled with diluent
3. Saline and plastic transfer pipette
4. Calibrated Westergren pipette (Dispette tube)
5. Sedimentation rate rack

Procedure

Safety Precaution
This procedure requires handling whole blood. Follow Standard Precautions, use appropriate PPE and dispose of sharps appropriately.

Note – Verify the procedure’s directions with those provided by the manufacturer of this product.

1. Remove the cap on the prefilled blue reservoir.
2. Using a disposable pipette, fill the vial to the indicated fill line with approximately 1 mls of well mixed whole blood to make the required 4:1 dilution. Be sure the blood mixture reaches the filling line.
3. Replace the blue cap carefully and securely. Gently invert several times to mix the blood and saline diluent. Using a sharpie, label the top of the vial with patient’s last name and ID number.
4. While holding filling reservoir firmly with one hand and the Dispette tube with the other hand positioned at 180 mm mark, twist the Dispette carefully into the blood/saline mixture in the reservoir.
5. Gently continue twisting the Dispette tube to the bottom of reservoir. Ensure that the blood level rising up the Dispette tube, reaches the zero (“0”) mark on the Dispette tube, but does not go past the zero mark.
6. Place the filled Dispette assembly in the sed rate rack provided by your instructor with the graduated portion of the tube facing outward. Ensure that the Dispette tube is straight up and down. The rack must be kept on a level surface and away from moving instruments or other sources of vibration.
7. Set a timer for exactly one hour.
8. After one hour, the result is read by determining exactly how many millimeters the red cells have fallen. Record the result as number of millimeters per hour.
9. Have the instructor look at your tube to make sure you are reading the value correctly at the end of the hour.
10. Read and record additional Sed Rate tubes provided by the instructor.
11. Record results in the appropriate place provided using appropriate units.
EXERCISE 9: ERYTHROCYTE SEDIMENTATION RATE (ESR) Study Questions

NAME _____________________________   DATE ____________________   _____ / 22 points

Directions: Unless otherwise stated, each question is worth one point.

1. State the principle of the ESR test.

2. State four patient factors that affect the ESR test. (2 points)
   a. 
   b. 
   c. 
   d. 

3. Define rouleaux and how it affects the ESR. (2 points)
   a. 
   b. 

4. Describe the stages that occur during the ESR by completing the following table. (3 points)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time frame and description of what is happening</th>
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<tbody>
<tr>
<td>First Stage</td>
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<tr>
<td>Second Stage</td>
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<tr>
<td>Third Stage</td>
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</table>

5. State the clinical significance of the ESR test.

6. List 4 reasons the ESR is performed. (2 points)
   a. 
   b. 
   c. 
   d. 
7. List 2 sample types that may be used for the ESR test.
   a. 
   b. 

8. Of the 2 sample types listed above, what advantage does one have over the other?

9. List 4 mechanical and technical factors that can affect the ESR. (2 points)
   a. 
   b. 
   c. 
   d. 

10. State the normal values for the Modified Westregren ESR for males and females. (2 points)
    a. 
    b. 

11. List at least four (4) sources of error that will adversely affect the ESR test. (4 points)
    a. 
    b. 
    c. 
    d. 

12. Thinking question: A patient had an ESR of 65mm/hour 1 month ago. Today, their ESR is 45mm/hour. Does today’s result represent a rising or falling ESR? Why? (1 point)
EXERCISE 9: ERYTHROCYTE SEDIMENTATION RATE (ESR)

Name____________________________ Date____________________________ Points: ____/20

<table>
<thead>
<tr>
<th>Specimen #1 (4 points possible)</th>
<th>Results – with correct units (2 points possible each)</th>
<th>Interpretation – Normal / Abnormal (2 points possible each)</th>
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</thead>
<tbody>
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<td>Patient Name</td>
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<tr>
<td>Patient ID</td>
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<td>Start time</td>
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<td>Stop time</td>
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<td>Specimen #2 (2 points possible)</td>
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<td>Specimen #3 (2 points possible)</td>
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Instructor signature: __________________________________________________________