EXERCISE 5: ERYTHROCYTES SEDIMENTATION RATE - ESR, SED RATE

Skills: 10 points

Objectives:
1. State the principle of the Erythrocytes Sedimentation Rate - ESR.
2. List two factors which may affect the results obtained in the ESR.
3. List one red cell and one plasma factor and how it will impact the results of the ESR.
4. List two samples which are acceptable for the ESR and state the advantage one has over the other.
5. List and describe the three stages which occur during the sedimentation of the red blood cells.
6. State the clinical significance of the ESR.
7. List three diseases which may have a markedly elevated ESR.
8. State the length of time a sample may be used for the ESR based on storage temperature.
9. State the normal values for the ESR for men and women using the proper units.
10. List nine sources of error which may cause erroneous results in the ESR.
11. Perform an ESR utilizing proper technique and equipment.

Discussion
The erythrocyte sedimentation rate (ESR) is a simple and inexpensive laboratory test. It is commonly used to assess the acute phase of the inflammatory response and to help diagnose conditions associated with acute and chronic inflammation, including infections, cancers, and autoimmune diseases. ESR is said to be nonspecific because increases do not tell the doctor exactly where inflammation is in the body or what is causing it, and also because it can be affected by other conditions besides inflammation. For this reason, ESR is typically used in conjunction with other tests.

The principle of the ESR is based on the fact that when well-mixed venous blood is placed in a vertical tube, the red blood cells (RBCs) will settle out of the plasma and fall towards the bottom of the tube and the pale yellow liquid (plasma) rises to the top. After 60 minutes, measurements are taken of the distance the red cells traveled to settle at the bottom of the tube.

The length of the fall of the top of the column of red blood cells in a given interval of time is the erythrocyte sedimentation rate - ESR. Normally red cells don't settle far toward the bottom of the tube, they fall slowly, leaving little clear plasma. Many diseases make extra or abnormal proteins (such as fibrinogen or immunoglobulins, which are increased in inflammation) that cause the red cells to move close together, stack up, and form a column (rouleaux). In a group, red cells are heavier and fall faster. The faster they fall, the further they settle, and the higher the ESR.

Erythrocyte sedimentation is affected by two major physiological factors: red cell surface charges and frictional forces around the red cell. The erythrocytes normally have net negative charges and, therefore, repel each other. High molecular weight proteins, especially when positively charged, increase viscosity and favor rouleaux formation and thus would raise the ESR. Rouleaux is a condition in which the RBCs clump together like stacks of coins. As the stack increases, it becomes heavier and tends to fall to the bottom faster. Fibrinogen, the most abundant acute phase reactant, has the greatest effect on the elevation of ESR when compared with other acute phase proteins. Paraproteins are positively charged molecules and when abundantly present as in multiple myeloma or Waldenstrom’s macroglobulinemia will increase the ESR levels by enhancing rouleaux formation and elevating plasma viscosity.

On the other hand, a change in the frictional forces around the red blood cell can affect the ESR. Anemia causes a falsely increased ESR because the change in the RBC to plasma ratio decreases the frictional forces.
which favors rouleaux formation, causing the RBCs to fall quickly. With the hematocrit reduced, the velocity of the upward flow of plasma is altered so that red blood cell aggregates fall faster. Macrocytosis with a small surface-to-volume ratio have charge relative to their mass and thus sediment more rapidly.

Some authors prefer to list red cell and plasma factors which affect the ESR:

<table>
<thead>
<tr>
<th>Factors Tending to Lower ESR</th>
<th>Factors Tending to Raise ESR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red Cell Factors</strong></td>
<td><strong>Red cell factors</strong></td>
</tr>
<tr>
<td>• polycythemia</td>
<td>• anemia</td>
</tr>
<tr>
<td>• abnormal red cells, eg spherocytosis, microcytosis, sickle cells</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Factors</strong></td>
<td><strong>Plasma Factors</strong></td>
</tr>
<tr>
<td>* cryoglobulins</td>
<td>* increased fibrinogen</td>
</tr>
<tr>
<td>* low fibrinogen</td>
<td>* increased globulins</td>
</tr>
<tr>
<td></td>
<td>* paraproteins</td>
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<tr>
<td></td>
<td>* low albumin</td>
</tr>
<tr>
<td></td>
<td>* cold agglutinins</td>
</tr>
<tr>
<td></td>
<td>* certain hyperlipidaemic states</td>
</tr>
</tbody>
</table>

*Mechanical and Technical factors* can also negatively affect the test. Proper technique and correct use of the equipment is critical for valid results. Care must be taken to follow procedural instructions exactly as temperatures which are too high or low, vibrations on the counter, unbalanced rack, improper dilution of blood, use of samples older than recommended can negatively impact the results.

The sedimentation rate of RBCs takes place in three stages:

1. *First Stage* - During the first 10 minutes rouleaux form, with slow sedimentation.
2. *Second Stage* - For the next 40 minutes cells settle rapidly, with most of the settling occurring during this period.
3. *Third Stage* - during the last 10 minutes, packing occurs, sedimentation rate is slow because of the accumulation of RBCs in the bottom of the tube.

**Sample**

Two samples are acceptable for the ESR, EDTA and the black stoppered tube. An EDTA sample is appropriate but requires dilution of the blood prior to testing. The black stoppered has the advantage of having the correct amount of anticoagulant and diluent so that the test can be performed directly from the blood in the tube. The black stoppered tube MUST BE completely filled. Using the black stoppered tube decreases the chances of error due to an improper dilution of the EDTA sample.

If the sample is stored at *room temperature* (20-25 C) the ESR should be set up within two hours of blood collection. EDTA specimens stored in the refrigerator at 4 C for 24 hours or less may be used.

**Clinical Significance**

The ESR is a nonspecific indicator of inflammation and necrosis. It reflects mainly the increase in production of a certain protein produced in response to necrosis or inflammation. These changes occur in many acute
and chronic infections, tumors and degenerative diseases autoimmune diseases such as rheumatoid arthritis.

Doctor’s do not base their decisions solely on ESR results. You can have a normal result and still have a problem. The use of the ESR at the present time is as an indication of the presence of active disease. This test may help detect the existence of disease but not its severity.

ESR is helpful in diagnosing two specific inflammatory diseases, temporal arteritis and polymyalgia rheumatica. A high ESR is one of the main test results used to support the diagnosis. A physician usually orders an ESR test (along with others) to evaluate a patient who has symptoms that suggest polymyalgia rheumatica or temporal arteritis, such as headaches, neck or shoulder pain, pelvic pain, anemia, unexplained weight loss, and joint stiffness. There are many other conditions that can result in a temporary or sustained elevation in the ESR. It is also used to monitor disease activity and response to therapy in both of these diseases.

*Moderately elevated ESR* occurs with inflammation, but also with anemia, infection, pregnancy, and old age.

A *markedly increased ESR* usually has an obvious cause, such as a marked increase in globulins that can be due to a severe infection or inflammation. The doctor will use other follow-up tests, such as cultures, depending on the patient’s symptoms.

Other conditions which may have a markedly elevated ESR include:

- *Rheumatoid arthritis*, an autoimmune disease causing severe inflammation of the joints.
- *Multiple myeloma* or *Waldenstrom’s macroglobulinemia* (tumors that make large amounts of immunoglobulins) typically have very high ESRs even if they don't have inflammation.
- *Polymyalgia rheumatica*
- *Temporal arteritis.*

Although a *low ESR* is not usually important, it can be seen with polycythemia (a condition where a patient makes too many red blood cells), with extreme leukocytosis (patient has too many white blood cells), and with some protein abnormalities. Some changes in red cell shape (such as sickle cells in sickle cell anemia) also lower the ESR.

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

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 protein which is actually produced and this test can detect and quantitate 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. 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. CRP is not affected by as many other factors as is ESR, making it a better marker of inflammation. However, because ESR is an easily performed test, many doctors still use ESR as an initial test when they think a patient has inflammation.

**Normal Values**

Adults (Westergren method):
* Men under 50 years old: less than 15 mm/hr.
* Men over 50 years old: less than 20 mm/hr.
* Women under 50 years old: less than 20 mm/hr.
* Women over 50 years old: less than 30 mm/hr.

Children (Westergren method):

* Newborn: 0 to 2 mm/hr.
* Neonatal to puberty: 3 to 13 mm/hr

**Sources of Error**

Sources of error which may adversely affect the results of the ESR include:

1. If the concentration of the anticoagulant is greater than recommended, the ESR will be erroneously high. This is especially critical if the black top, which contains the diluent for the test, is used. EDTA samples may be used for the ESR and are diluted prior to performing the test.
2. If the ESR stands for more than 60 minutes, the results will be falsely elevated. If the test is timed for less than 60 minutes, 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. The tubes must be maintained 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 in the blood tube when filling will lead to erroneous results.
6. Fibrin clots present in the blood invalidate the test results.
7. If the sample is stored at room temperature (20-25 C) the ESR should be set up within two hours of blood collection. EDTA specimens stored in the refrigerator at 4 C for 24 hours or less may be used.
8. If sample has been stored in the refrigerator allow to warm up to room temperature before testing.
9. The sample must be well mixed prior to use.

**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 5: ERYTHROCYTE SEDIMENTATION RATE**

**Specimen:**

1. Fresh whole blood collected in EDTA anticoagulant
2. Check all specimens for clots with applicator sticks
3. Specimen should be assayed within 2 hours if kept at room temperature or 24 hours if refrigerated.
**Equipment:**

1. Sed rate tube
2. Reservoir prefilled with diluent
3. Disposable pipettes
4. Sed rate rack

**Procedure:**

1. Remove the blue cap on the prefilled reservoir.
2. Using a disposable pipette, fill the vial to the indicated fill line with approximately 0.8 mls of well mixed whole blood to make the required 4:1 dilution.
3. Replace the piercable stopper and gently invert several times to mix.
4. Insert sed rate tube through the piercable stopper using a twisting pushing motion into the C and autozero to the “0” position.
5. Place the vial in its rack on a level surface.
6. Set the time for exactly one hour.
7. After one hour, read the numerical results of the ESR in **milimeters** at the plasma meniscus.
8. Record the results on the log sheet.

**EXERCISE 5: ERYTHROCYTE SEDIMENTATION RATE**

Name _________________________________

<table>
<thead>
<tr>
<th>Patient Name</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient ID Number</td>
<td></td>
</tr>
<tr>
<td>Start Time</td>
<td></td>
</tr>
<tr>
<td>Stop Time</td>
<td></td>
</tr>
<tr>
<td>Results (use correct units)</td>
<td></td>
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</tbody>
</table>

Do these results fall within the normal range (circle one): **YES NO**
EXERCISE 5: ERYTHROCYTE SEDIMENTATION RATE STUDY QUESTIONS

Name ____________________________ Date ___________________

Points: 17

1. State the principle of the Erythrocyte Sedimentation Rate (ESR). 1 point

2. Within what time frame of sample collection should the ESR be set up? Be sure to state the specific temperature of storage for both room temperature and refrigeration. (2 Point)

3. State the two factors which affect the sedimentation rate. (2 points)
   A.
   B.

4. Fill in the following chart (2 points) by listing one factor which may affect each item listed.

<table>
<thead>
<tr>
<th></th>
<th>Lower ESR</th>
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<tbody>
<tr>
<td>Red Cell Factors</td>
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<tr>
<td>Plasma Factors</td>
<td></td>
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</tbody>
</table>

5. Describe the three stages which occur during the sedimentation rate (1.5 points).
   A.
   B.
   C.

6. List 2 sample types which may be used for the ESR. (1 point)
7. Of the 2 samples which may be appropriate for testing state the advantage that one has over the other. (1 point)

8. State the clinical significance of the ESR (1 point)

9. List three diseases/conditions which may have *markedly increased* ESR results (1.5 points).
   a. 
   b. 
   c. 

10. List four sources of error in the ESR test which may adversely affect the results (2 points).
    a. 
    b. 
    c. 
    d. 

11. State the normal values for the Modified Westergren ESR for males and females (2 point).