1. **State the principle of the erythrocyte sedimentation rate (ESR).** (2 points)
   The principle of the ESR is based on the fact that when non-coagulated blood is placed in a vertical tube, the red blood cells (RBCs) settle out of the plasma due to gravitational force and fall towards the bottom of the tube. The specific gravity of erythrocytes (1.095 and highest among the blood constituents), their percent composition (4% by volume), as well as their biconcave shape facilitate their sedimentation rate.

2. **How do you measure the ESR rate?** (2 points)
   For an ESR test, an anti-coagulated blood sample (venous blood drawn from a person) is placed into a thin upright tube, either a Westergren tube or Wintrobe tube, and the blood is left undisturbed (with no vibrations) for an hour to sediment under the influence of gravity. The resulting clear plasma on top of the sedimented erythrocytes is measured in units of height (millimeters) per hour.

3. **List three factors that may affect the ESR.** (1.5 points)
   1. Fluid factors
   2. Particle factors
   3. Mechanical factors

4. **Describe the three stages that occur during the sedimentation rate.** (1.5 points)
   1. Particles begin to join; the rate is very low
   2. Particles begin to separate from the fluid; the rate is somewhat appreciable
   3. Accumulation of the sediment particles on the tube bottom; the sedimentation rate is very slow

5. **State the clinical significance of the ESR.** (5 points)
   The ESR is a simple, non-specific screening test that indirectly measures the presence of inflammation or infection, or other blood or body disorders. Non-specific means the test does not identify the source of the problem or illness that is causing the inflammation.

6. **List the diseases/conditions that may have higher-than-normal ESR results.** (4 points)
   1. Inflammation
   2. Infection
   3. Cancer
   4. Anemia
   5. Autoimmune disorders such as
      - Temporal arteritis
      - Polymyalgia
      - Rheumatoid arthritis

7. **List the diseases/conditions that may have lower-than-normal ESR results.** (4 points)
   1. Polycythemia
   2. Abnormal proteins
   3. Sickle-cell anemia
   4. Leukocytosis
For the following three questions, you may use Internet research to prepare your answers.

8. **Explain why the erythrocyte sickle shape lowers the erythrocyte sedimentation rate?** (10 points)

In ESR we are measuring the height of the clear plasma on top of the sediment, which is greater if the erythrocytes have settled at the bottom as a dense, compact solid mass.

We know the sedimentation process is composed of three major stages:
- Particles begin to join; the rate is very low
- Particles begin to separate from the fluid; the rate is somewhat appreciable
- Accumulation of the sediment particles in the tube bottom; the sedimentation rate is very slow

These three stages reveal that the major influence on the overall rate of sedimentation of erythrocytes suspended in plasma is the degree to which they aggregate with one another, within a layer and in between the layers. Two particle parameters become very important in aggregation; they are: 1) shape and 2) mass.

Shape influences the aggregating ability, while mass influences whether a material floats or sinks, and the gravitational pull experienced by the particles.

1) Rounded or spherical particles tend to pack together tightly to create dense sediments. By contrast, angular particles have low sphericity and pack less-effectively, leaving more interstitial spaces that are occupied by the solvent, making them less able to come out of the solvent.

2) Particles with less mass experience less gravitational pull, and also have more tendency to float (mass of water displaced is more than the mass of the particle).

A sickle cell has less mass because some portions of the erythrocyte are missing and it has an angular shape; therefore, it does not settle out of the plasma, resulting in ESR values that are less than normal values.

![Rounded shape = more sphericity](image1.png)

![Angular shape = less sphericity](image2.png)

9. **Discuss why fibrinogen and immunoglobulins increase the erythrocyte sedimentation rate?** (10 points)

We are aware that fluid factors influence the erythrocyte sedimentation rate. Plasma is a multi-component fluid; besides the erythrocytes, plasma holds a number of materials, both dissolved and dispersed. Each material in plasma has a function. Plasma proteins constitute an important component of plasma. Plasma proteins are colloidal in nature and have a general function, namely buffering or maintaining the acid-base balance. In addition, each plasma protein has a specific function.

Two very important plasma proteins are fibrinogen and immunoglobulins. Immunoglobulins combine with foreign antigens and remove them. The function of fibrinogen is in the clotting process (hemostasis). Both these proteins are binding proteins.

Generally, the immunoglobulin level increases if an infection exists. An elevated level of fibrinogen results from blood vessel constrictions (atherosclerosis). At elevated levels, the fibrinogen and immunoglobulin come in between the erythrocytes, which leads to greater cohesion of erythrocytes because they serve like glue and accelerate the aggregation of erythrocytes.
The erythrocytes settle out of plasma quickly and settle down and quickly thicken. So, at the end of one hour in the ESR test, the height of clear plasma on top of the sediment is greater. It has been proven that when erythrocytes sediment, they stack like coins (called rouleaux) and fall. Then several such stacks of erythrocytes further aggregate and thicken to form a compact dense sediment enabling even more plasma to be clear.

An illustration of the sedimentation of erythrocytes aided by the binding effect of fibrinogen and immunoglobulins. (left) Falling sediment; (middle) sediment compaction; (right) thickened sediment.

10. Discuss why anemia increases the erythrocyte sedimentation rate? (10 points)
   In anemia, a reduced level of erythrocytes exists, as compared to the normal value. That suggests a situation of unhindered settling with a lower ESR value. Thus, it appears strange when we find increased ESR in the case of anemia.

   Normally at reduced concentration, erythrocytes do not have the favorable condition to collide with each other to aggregate and sediment. However, three plasma factors that are at very low levels in anemia cause high ESR. They are:

   (1) Serum ferritin
   (2) Serum iron
   (3) Iron-binding capacity

   Serum ferritin is a colloidal protein; as its concentration decreases, plasma becomes less viscous. Similarly, less serum iron causes plasma to be dilute and less viscous. Poor iron-binding capacity is an indication that serum iron and ferritin do not bind, which again indicates that the plasma is dilute and less viscous. When the plasma is dilute and less viscous, it does not cause resistance to the fall of the erythrocytes.