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Theoretical part
Erythrocyte sedimentation rate

Blood plasma

Blood plasma is an amber solution containing dissolved organic as well as inorganic compounds. Plasma represents 5% of the body mass, which stands for 3.5l in an average man weighing 70 kg. Blood plasma is obtained by the centrifugation of anticoagulated blood, thus it contains clotting proteins in comparison to the blood serum.

The organic compound of blood plasma is made up from proteins, glucose, hormones, vitamins, enzymes etc. The plasmatic proteins can be further divided into an albumin, globulin and fibrinogen fraction. The physiological concentration range of the plasma proteins is 65–85 g/l, but the vast majority is represented by albumin (25–51 g/l). The capillary wall is, under physiological conditions, a completely impenetrable barrier for the proteins dissolved in plasma and hence an osmotic pressure of around 25 mmHg is created. Furthermore, the proteins represent a valuable buffer system maintaining the pH of blood plasma in the very narrow range 7.36-7.44, a fast source of amino acids, they play an important role in immune reactions, coagulation, transportation of substances such as fatty acids, steroid hormones, bilirubin, ions, metals, drugs and so forth.

The inorganic compound of blood plasma consists of dissolved ions.

Ion	Concentration
Sodium	135–145 mmol/l
Potassium	3.7–5.1 mmol/l
Chloride	96–108 mmol/l
Calcium	2–2.6 mmol/l
Calcium ionized	1.16–1.32 mmol/l
Inorganic phosphates	0.65–1.61 mmol/l
Magnesium	0.78–1.03 mmol/l

Erythrocyte sedimentation rate (FW)

The erythrocyte sedimentation rate (Fahraeus-Westergren) is a nonspecific laboratory method providing information for the caregiver that something is going on in the patient's body. Sedimentation is a process of unsolvable particles settling in the suspension. Its counterpart is suspension stability, which is represented by zeta potential, the negative charge on the surface of erythrocytes determined by sialic acids bound on glycophorin which repel erythrocytes away from each other. This procedure is traditionally performed as anticoagulated blood placed in an upright tube and the erythrocyte sedimentation rate is measured after one hour.

Factors influencing the erythrocyte sedimentation rate:

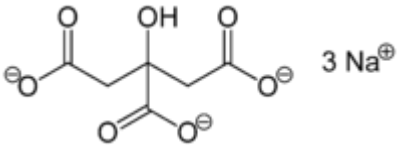
- Red blood cell diameter – the bigger the red blood cells are, the faster sedimentation is
- The number of erythrocytes – more erythrocytes means greater zeta potential and higher suspension stability

- The plasmatic proteins
    - Albumin – possess negative charge, and this leads to higher suspension stability
    - Fibrinogen – is negatively as well as positively charged and so suspension stability is decreased
    - Immunoglobulins – also hold negative and positive charge and hence lower suspension stability

Factors disrupting the zeta potential lead to rouleaux production. Roleaux sediments faster because of increased ratio of volume/surface area.

Erythrocyte sedimentation rate is measured in anticoagulating blood produced by addition of:

- Natrium citrate – binding Ca^{2+}



- EDTA - Ethylenediaminetetraacetic acid – this also acts as a chelating agent
- Natrium oxalate – the same as EDTA
- Heparin – activation of antitrombin III

Physiologic values:

- Man – 2–8 mm/h.
- Woman – 7–12 mm/h.
- Newborn – 2 mm/h
- Toddler – 4–8 mm/h.

→ the gender differences are caused by sex hormones; testosterone increases the erythropoietin production in kidneys → more erythrocytes

Pathologies:

- Increased erythrocyte sedimentation rate:
    - Pregnancy
    - Macrocytosis
    - Infection
    - Tumours – myeloma
    - Inflammations
    - Necrosis (infarction, trauma)
    - Relative/absolute albumin loss (nephrotic syndrome)
- Decreased erythrocyte sedimentation rate
    - Misshaped erythrocytes – spherocytosis
    - Polycythemia vera
    - Leucocytosis
    - Dysproteinaemia – hypofibrinogenaemia, hypogammaglobinaemia
    - Dehydration

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Protocol

Erythrocyte sedimentation rate

Methods

Equipment: Stand with a set of sedimentation pipettes, rubber cups, sucking rubber cylinders, six samples of blood:

- anti-coagulated human blood with full plasma
- anti-coagulated bovine blood with full plasma
- anti-coagulated horse blood with full plasma
- anti-coagulated human blood, the plasma of which is totally replaced by physiological solution
- anti-coagulated horse blood, the plasma of which is totally replaced by physiological solution
- anti-coagulated human blood with low R.B.C. (Ht = 0.29)

Procedure:

1. Mix well the anti-coagulated undiluted blood (**use gloves!**) and pour 2–3 ml into the first rubber cup which is placed in the stand in such way that the tip of the sedimentation pipette is exactly in the centre.
2. The rubber cylinder is put on the upper end of the pipette and used to suck up the blood. By slightly pressing the cylinder, a small amount of air is expelled from the pipette, the lower end of which is then immersed into the cup filled with blood. Release gradually the cylinder to suck blood up to the mark 0 (height 200 mm). In that moment, press the pipette down towards the bottom of the rubber cup. Then, with the hand which was used to suck up the blood, fix the pipette in this position by tightening its screw. In the same way, the remaining two pipettes are filled with the other samples.
3. Read the sedimentation values every 15–20 minutes so that you obtain at least 3 values (in clinical practice, values after 1 and 2 hours are recorded).

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Results

Graph of erythrocyte sedimentation rate (choose different colours for each specimen):

Time (min)	15	30	45	60	75	90	105	120
human blood with full plasma								
Human erythrocytes with physiol. solution								
bovine blood with full plasma								
horse blood with full plasma								
human blood with low R.B.C.								
horse erythrocytes with physiol. solution								

List all the factors influencing the erythrocyte sedimentation rate:

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Conclusion

Explain the differences between the blood samples you worked with.

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