Physiology of Blood II
Practicals

• Erythrocyte sedimentation rate
• Osmotic resistance of erythrocytes
• Determination of blood density
• Count of erythrocytes
Erythrocyte sedimentation rate (ESR)

• ESR determination is a **simple** laboratory test
  • FW (abbreviation, means Fahreus-Westergren)

• **Serum proteins** play a crucial role in increased ESR value

• The test measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under **the influence of gravity**.

• An elevated value remains a **nonspecific** finding.

• The most satisfactory method of performing the test was introduced by Westergren in 1921
  • 4 minute test - rapid
  • Wintrobe method - narrow tubes used
ESR - principle

Gravitational force

ESR tube normal

ESR tube increased

- erythrocytes
- proteins - albumin
- globulins - fibrin
Factors That May Influence ESR

Factors that increase ESR
- Old age
- Female
- Pregnancy
- Anemia
- Red blood cell abnormalities
- Macroglossia
- Technical factors
- Dilutional problem
- Increased temperature of specimen
- Tilted ESR tube
- Elevated fibrinogen level

Factors that decrease ESR
- Extreme leukocytosis
- Polycythemia
- Red blood cell abnormalities
- Spherocytosis
- Acanthocytosis
- Microcytosis
- Technical factors
- Dilutional problem
- Inadequate mixing
- Clotting of blood sample
- Short ESR tube
- Vibration during testing

Factors with no clinically significant effect or questionable effect
- Obesity
- Body temperature
- Recent meal
- Aspirin
- NSAIDs

Factors with no clinically significant effect or questionable effect
- Extreme leukocytosis
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Protein abnormalities
- Hypofibrinogenemia
- Hypogammaglobulinemia
- Dysproteinemia with hyperviscosity state

Reference Ranges for the ESR in Healthy Adults

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Upper limit of reference range (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 50 years</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0 to 15</td>
</tr>
<tr>
<td>Women</td>
<td>0 to 20</td>
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<tr>
<td>Age &gt; 50 years</td>
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<td>0 to 30</td>
</tr>
</tbody>
</table>

ESR = erythrocyte sedimentation rate.

ESR [mm/hrs]= \frac{(Age \text{ (years)} + 10 \text{ (if women)})}{2}
ESR - how does it look
Osmotic resistance

• Osmosis
  • cell membranes are semipermeable
  • osmotic gradients makes water/diluent to move from environment with ↓ concentration of solutes to environment with ↑ concentrations of solutes
  • osmotic pressure depends upon the difference between the concentration of non-diffusible ions on each side of the membrane

• Saline/Physiologic solution
  • 0.9% NaCl solution, or 0.9 g of salt into 100ml of water
  • Isotonic = osmotic equilibrium
  • No net influx/efflux of water through membrane, if cell resides in such environment

• The ability of the RBC to resist the osmotic power before the cell will lyse
  • hypertonic solutions = hemolysis
  • hypotonic solutions = hemolysis

• The older the erythrocyte, the lower the osmotic resistance, the sooner hemolysis will occur
Osmotic resistance

• Hemolysis values:
  • Partial hemolysis
    • Minimal osmotic resistance
    • 0.39% to 0.45% salt solution
  • Complete hemolysis
    • Maximal osmotic resistance
    • 0.30% to 0.33% salt solution
  • Resistance width
    • Difference between partial and complete hemolysis

• in 24 hours at 37 °C (98.6 °F)
Results

• $\uparrow$ osmotic resistance $\Rightarrow$ $\downarrow$ osmotic fragility
  • iron deficiency anemia (microcytic anemia)
  • thalassemia
  • sickle cell anemia
  • obstructive jaundice
  • after splenectomy

• $\downarrow$ osmotic resistance $\Rightarrow$ $\uparrow$ osmotic fragility
  • hereditary spherocytosis
  • congenital hemolytic anemia
  • spherocytosis other than hereditary
Determination of specific gravity/blood density

• **Specific gravity**
  – total mass [weight]/equal volume of water [weight]; $\rho = \frac{\Sigma M}{\Sigma V}$
  – Indirect method measurement by Van Slyke copper sulphate method

• **Blood density depends on**
  • hematocrite (No. of RBC, hemoglobin content)
  • plasma protein concentration
  • water content of blood

• **Good index of hemoglobin content**
  • fast screening of blood donors
  • during war times or catastrophes, fast method to assess needs for blood transfusion
Determination of blood density

The density of whole blood 1048-1066 kg/m³

Factors ↑ specific density
- high altitude
- newborns and infants
- excess sweating
- diarrhea
- vomiting
- polycythemia

Factors ↓ specific density
- pregnancy
- water intake (excess)
- nephrotic syndrome (overhydration)
Leukocyte count

• As ERY count, only....

• Turk solution
  • 1-2% acetic acid (destroys ERY)
  • Gentian violet (stains LEU nuclei)

• Count in 50 squares

• Divide by 10 and multiply by $10^9$, get No. of Leu in L of blood

• Normal values $3.5 - 9 \times 10^9$/L blood
Leukocyte count
Questions?

• Reading for next practicals: Blood Groups and Red Cell Antigens
  • [https://www.ncbi.nlm.nih.gov/books/NBK2261/?term=blood%20groups](https://www.ncbi.nlm.nih.gov/books/NBK2261/?term=blood%20groups)
    • Chapter 1, 2
    • Chapter 3 (until Transfusion reactions)
    • Chapter 4, 5, 6, 7 and 9
  
  • If interested: 8 and 11

Go to google.com, insert all key words: Blood groups and red cell antigen
It is the first link through NCBI Bookshelf