Experiment no.: 4.

**Experiment name:** Determination of HDL-cholesterol in the blood serum.

**The aim of the Experiment:**

Determination of HDL- in the blood using DIFFERENTIAL PRECIPITATION

*Enzymatic colorimetric test.*

This technique uses a separation method based on the selective precipitation of apolipoprotein B-containing lipoproteins (VLDL, LDL and (a)Lpa) by phosphotungstic acid/MgCl₂, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant.

**Equipment and martials used in the Experiment:**

I. **Precipitation**

- Dilutor and pipettes.
- Centrifuge tubes (13 x 100 m/m). – Vortex mixer.
- Desktop centrifuge.

II. **Colorimetry**

- Kit for measuring Total Cholesterol.
- Constant temperature incubator set at 37°C.
- Photometer or colorimeter capable of measuring absorbance at – 500±10nm.

**Property of the machine:**

Normal UV-Vis spectrophotometer:

**Machine usage:**

- Wavelength set up step.
- Blank against the solvent solution using a proper cuvette.
- Reach O.D.

**Experiment procedure or protocol:**

**REAGENT COMPOSITION**

**R1 Precipitating reagent.** Phosphotungstic acid 0.63 mmol/L, magnesium chloride 25 mmol/L. Stabilizers.
**Cholesterol standard.** Cholesterol 50 mg/dL (1.3 mmol/L). Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 1951a. Not included.

**R2 Cholesterol MR.**

I. *Precipitation*
1. Bring reagents and samples to room temperature.

2. Pipette into labelled centrifuge tubes:

![Ratio Calculation](image)

3. Vortex and allow to stand for 10 minutes at room temperature. 4. Centrifuge for 10 minutes at 4000 r.p.m., or 2 minutes at 12000 r.p.m.

5. Separate off the clear supernatant within 2 hours.

*In case of turbid supernatants caused by elevated triglycerides (>350 g/dL) the sample should be diluted 1:2 with saline and steps 2,3,4 and 5 repeated. Multiply the result of the colorimetry by 2.*

II. *Colorimetry*

1. Bring the Cholesterol MR Monoreagent and the cholesterol standard (50 mg/dL) of the kit to room temperature

2. Pipette into labelled tubes:

<table>
<thead>
<tr>
<th>TUBES</th>
<th>Blank</th>
<th>Sample Supernat</th>
<th>Standard Supernat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoreagent Supernate Standard</td>
<td>1.0 mL</td>
<td>1.0 mL 50 μL</td>
<td>1.0 mL – 50 μL</td>
</tr>
</tbody>
</table>

3. Mix and let the tubes stand for 10 minutes at room temperature or 5 minutes at 37 °C.

4. Read the absorbance (A) of the supernatant and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

**CALCULATIONS**

\[
\text{A Supernatant/ A Standard} \times \text{C Standard} = \text{mg/dL HDL-Cholesterol}
\]

If results are to be expressed as SI units apply: mg/dL x 0.0259 = mmol/L
Experiment data and results:

Clinical values of HDL-Cholesterol used to classify risk groups.

<table>
<thead>
<tr>
<th>Cholesterol from lipoproteins of high density</th>
<th>RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>&gt; 55 mg/dL ( &gt; 1.42 mmol/L)</td>
<td>Low</td>
</tr>
<tr>
<td>35-55 mg/dL (0.90-1.42 mmol/L)</td>
<td>Moderate</td>
</tr>
<tr>
<td>&lt; 40 mg/dL ( &lt; 1.04 mmol/L)</td>
<td>High</td>
</tr>
<tr>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>&gt; 65 mg/dL ( &gt; 1.68 mmol/L)</td>
<td>Low</td>
</tr>
<tr>
<td>45-65 mg/dL (1.16-1.68 mmol/L)</td>
<td>Moderate</td>
</tr>
<tr>
<td>&lt; 45 mg/dL ( &lt; 1.16 mmol/L)</td>
<td>High</td>
</tr>
</tbody>
</table>

Conclusion:

- Where is HDL synthesized from?
- How does it affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?