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 apoliprotein B-containing lipoproteins (VLDL, LDL and (a)Lpa) by phosphotungstic acid/$MgCl_2$, 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 ºC.
− 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:

Sample or Standard	0.2 mL
Precipitating reagent	0.4 mL

Ratio $\frac{\text{Sample}}{\text{Reagent}} = \frac{1}{2}$

Dil. factor = 3

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:

TUBES	Blank	Sample Supernat	Standard Supernat
Monoreagent Supernate Standard	1.0 mL – –	1.0 mL 50 µL –	1.0 mL – 50 µL

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

$A_{\text{Supernatant}} / A_{\text{Standard}} \times C_{\text{Standard}}$ = 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.		
Cholesterol from lipoproteins of high density		RISK
Men	> 55 mg/dL (> 1.42 mmol/L)	Low
	35-55 mg/dL (0.90-1.42 mmol/L)	Moderate
	< 40 mg/dL (< 1.04 mmol/L)	High
Women	> 65 mg/dL (> 1.68 mmol/L)	Low
	45-65 mg/dL (1.16-1.68 mmol/L)	Moderate
	< 45 mg/dL (< 1.16 mmol/L)	High

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?