Determination of lipid profile
Part 2

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C- Determination of blood High Density Lipoprotein (HDL).

It is Precipitation dependent method.

Meaning: in the circulation there are five type of Lipoproteins (HDL, LDL, VLDL, Chylomicron and ILD). The measurement method is rely on measure the cholesterol contain in the blood. As all the lipoproteins mentioned above has a cholesterol in it, we need to eliminate the undesirable lipoproteins to be able to measure the desirable lipoprotein.

PRINCIPLE OF THE METHOD.

The very low density (VLDL), Chylomicron and low density (LDL) lipoproteins from serum or plasma are precipitated by phosphor tungstate in the presence of magnesium ions. After centrifugation the supernatant contains high density lipoproteins (HDL). The HDL cholesterol fraction is determined using the total cholesterol enzymatic reagent.

CLINICAL SIGNIFICANCE

HDL particles carry cholesterol from the cells back to the liver. HDL is known as “good cholesterol” because high levels are thought to lower the risk of heart disease. A low HDL cholesterol levels, is considered a greater heart disease risk.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R Precipitating Reagent	Phosphotungstic acid Magnesium chloride	14 mmol/L 2 mmol/L
Optional STD (Note 2) **Optional reagent**	HDL Aq. Prim. Std. Cholesterol CHOD-POD	50 mg/dL

SAMPLES

Serum or plasma1: Free of hemolysis. Removed from the blood clot as soon as possible.
Stability : HDL Cholesterol is stable for 7 days at 2-8ºC .

PROCEDURE

Precipitation

1. Pipette into a centrifuge tube:

R (µL)	100
Sample (mL)	1,0

2. Mix well; allow to stand for 10 min at room temperature.
3. Centrifuge at 4000 r.p.m. for 20 min or 2 min at 12000 r.p.m.
4. Collect the supernatant and proceed it as a sample in the total cholesterol determination.

PROCEDURE

1. Assay conditions:
   Wavelength:. . . . . .. 505 nm (500-550)
   Cuvette: . . . . .1 cm light path
   Temperature:. . . . . .37ºC /15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette

	Blank	Standard	Sample
WR (mL)	1,0	1,0	1,0
Standard[(Note 1,3)] (μL)	--	10	--
Sample (μL)	--	--	10

4. Mix and incubate for 5 min. at 37ºC or 10 min. at room temperature.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 60 minutes.

CALCULATIONS

$$\frac{\text{(A) Sample - (A) Blank}}{\text{(A) Standard - (A) Blank}} \times 50 \quad \text{(Standardconc.)} = \text{mg/dL cholesterol in the sample}$$

REFERENCE VALUES.

HDL-cholesterol:

	Men	**Women**
Lower risk	> 55 mg/dL	> 65 mg/dL
Standard risk	35-55 mg/dL	45-65 mg/dL
Increased risk	< 35 mg/dL	< 45 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

D- Calculation of LDL-cholesterol (Friedewald)

LDL cholesterol= total cholesterol-(VLDL cholesterol +HDL cholesterol).

REFERENCE VALUE

Recommended value	< 100 mg/dL
Low Risk	130-159 mg/dL
High Risk	> 160 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

E. Calculation of VLDL

VLDL= TG/5 mg/dl

REFERENCE VALUE: < 30 mg/dl

Q/ What is the serum cholesterol levels in patient sample if we know the following information

1- patient tube Absorbance (A) = 40
2- Standard tube Absorbance (A)= 20
3- Blank Absorbance(A) =2
4- The concentration of standard is 200 mg/dl

Answer/

(40-2)/(20-2) x 200 = 422.22 mg/dl

Q/ what is the level of LDL and VLDL for patient admitted to CCU if we know the following

1. Total cholesterol level 220 mg/dl
2. TG level 300 mg/dl
3. HDL level 48 mg/dl

Answer/

LDL-c = total cholesterol-(VLDL cholesterol +HDL cholesterol).

VLDL = TG/5 = 300/5= 60 mg/ dl

LDL-c= 220 – (60 + 48) = 112 mg/dl

Thank you for your attention