Determination of lipid profile

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I will cover this topic in two lectures.

Lecture 1: Determination of blood Cholesterol and Triglyceride
Lecture 2: Determination of blood HDL and LDL

A- Determination of blood Total Cholesterol

PRINCIPLE OF THE METHOD

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in the sample.

Clinical Significance.

Cholesterol is a fat-like substance that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones.

The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipemia.

High blood cholesterol is one of the major risk factors for heart disease.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	PIPES pH 6,9 Phenol	90 mmol/L 26 mmol/L
R 2 Enzymes(Note 2)	Cholesterol esterase (CHE) Cholesterol oxidase (CHOD) Peroxidase (POD) 4 – Aminophenazone (4-AP)	300 U/L 300 U/L 1250 U/L 0,4 mmol/L
CHOLESTEROL CAL	Cholesterol aqueous primary standard 200 mg/dL Contains Triton X-114 10-15%	

PREPARATION

Working reagent (WR): Dissolve ( ) the contents of one vial R 2 Enzymes in one bottle of R 1 Buffer.

Cap and mix gently to dissolve contents.

(WR) is stable: 4 months at 2-8ºC or 40 days at 15-25ºC.

Avoid direct sunlight.

Signs of reagent deterioration:

- Presence of particles and turbidity.

SAMPLES

Serum or plasma: Stability of the sample for 7 days at 2-8ºC or freezing at –20ºC will keep samples stable for a 3 months.

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

$$\frac{\text{(A) Sample - (A) Blank}}{\text{(A) Standard - (A) Blank}} \times 200\ (\text{Standardconc.}) = \text{mg/dL cholesterol in the sample}$$

REFERENCE VALUES

Less than 200 mg/dL	Normal
200-239 mg/dL	Borderline
≥ 240 mg/dL	High

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

B- Determination of blood Triglycerides

PRINCIPLE OF THE METHOD

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase (GK) and ATP. Glycerol-3- phosphate (G3P) is then converted by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2).
In the last reaction, hydrogen peroxide (H2O2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerolkinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample.

CLINICAL SIGNIFICANCE

The measurement of the concentration in blood triglycerides is important for the diagnosis and the follow-up of hyperlipidemia. Its increase can be of genetic origin or secondary to other metabolic disorders such as: diabetes mellitus, hyper and hypothyroidisms, hepatic diseases, acute and chronic pancreatitis, nephrosis. A rise in triglycerides also represents an atherogenic risk factor. It is responsible for the opalescence, or even the cloudiness of the serum. Corticoids and oestrogen/progestin treatments can also aggravate hypertriglyceridemia.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	GOOD pH 7,5 p-Chlorophenol	50 mmol/L 2 mmol/L
R 2 Enzymes (Note 2)	Lipoprotein lipase (LPL) Glycerolkinase (GK) Glycerol-3-oxidase (GPO) Peroxidase (POD) 4 – Aminophenazone (4-AP) ATP	150000 U/L 500 U/L 2500 U/L 440 U/L 0,1 mmol/L 0,1 mmol/L
TRIGLYCERIDES CAL	Triglycerides aqueous primary standard 200 mg/dL	

PREPARATION

Working reagent (WR): Dissolve the contents of one vial R 2
Enzymes into one bottle of R 1 Buffer.
Cap and mix gently to dissolve contents.
WR stability: 6 weeks at 2-8ºC or 1 week at room temperature (15-25ºC).

Signs of reagent deterioration:
- Presence of particles and turbidity.

SAMPLES

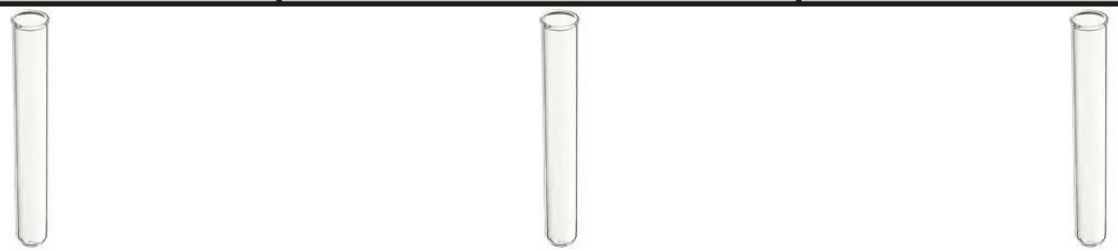
Serum or heparinized or EDTA plasma[1]. Stability of the sample: 5 days - . at 2-8ºC. And 3 months at -20 ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . .505 nm (490-550)
   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 30 minutes.

CALCULATIONS

$$\frac{\text{(A) Sample - (A) Blank}}{\text{(A) Standard - (A) Blank}} \times 200\ (\text{Standard conc.}) = \text{mg/dL triglycerides in the sample}$$

REFERENCE VALUES

Men: 40 – 160 mg/dL
Women: 35 – 135 mg/dL

Each laboratory should establish its own normal ranges for the population that it serves.

Thank you for your attention