Quantitative Determination of Blood Total Cholesterol

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Introduction

Quantitative Determination of Blood Total Cholesterol is considered one of the most commonly performed procedures in clinical biochemistry laboratory. Cholesterol is a type of lipids. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. It also serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D. The collected blood specimen for cholesterol estimation should be whole blood or serum or plasma without hemolysis and should obtained from the patient in fasting state (no food or drink for 8-12 hours).

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Clinical Significance

Total cholesterol assay which associated to assays of other lipids in serum is used in the diagnosis of hyperlipidemia, increased levels are also seen in hepatic and thyroid disorders.

Total cholesterol assay associated to triglycerides (TG's) HDL-Cholesterol and LDL-Cholesterol determination is useful in the prediction of coronary heart diseases (CHD's).

So, this assay is used in the diagnosis and treatment of atherosclerotic diseases. Hypercholesterolemia can also be observed in certain cases of diabetes. Secondary disorders that elevate cholesterol levels should be ruled prior to initiating therapy with cholesterol-lowering drugs.

Sample Handling

Samples for Total Cholesterol analysis should be delivered to the laboratory as soon as possible after being drawn from the patient to separate the blood serum within 2hours for analysis. Oxalate, Fluoride and Citrate tubes were not used in blood collection in this assay. Cholesterol in the blood serum is stable for 5-7 days at 2-8 °C, 3 months at -20 °C and many years at -70 °C but avoiding repeated freezing and thawing.

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Principle

Total Cholesterol is determined after enzymatic method in the presence of cholesterol esterase (CE) and cholesterol oxidase (CO). The hydrogen peroxide formed reacts, under catalysis of peroxidase (POD), with phenol and Para-aminoantipyrine (PAP) to form a pink quinoneimine dye as indicator.

$$\text{Cholesterol esters} \xrightarrow{CE} \text{Cholesterol + Free Fatty Acids (FFA's)}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Gluconic acid} + H_2O_2$$

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

Reagents

1- Buffer Reagent (Vial R1): Phosphate Buffer (100 mmol/L), Chloro-4-phenol (5 mmol/L), Sodium Cholate (2.3 mmol/L) and Triton x 100 Preservative (1.5 mmol/L).

2- Enzymes (Vial R2): CO (≥100 IU/L), CE (≥170 IU/L), POD (≥1200 IU/L), PAP (0.25 mmol/L) and PEG 6000 (167 µmol/L).

2- Standard Concentration Solution of Cholesterol (200 mg/dL).

Reagent Preparation

Working Reagent Solution was prepared by mixing and dissolving the content of Vial R2 in the content of Vial R2 and store it at 2-8 °C away from light.

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Procedure

1- Collect blood specimen from patients in serum blood tubes and leave it to coagulate for 20 minutes.
2- Separate serum sample from the blood by centrifuging at 3000-4000 RPM for 10-20 minutes.
3- Collect the serum in labeled tubes.
4- Three sets of tubes were prepared as below:

Solutions / Tubes	Blank	Standard	Serum
Working Reagent	**1 mL**	**1 mL**	**1 mL**
Standard	**-**	**10 µL**	**-**
Sample (Serum)	**-**	**-**	**10 µL**

5- All tubes were mixed well by vortex and incubated them for 5 minutes at 37ºC.
6- The absorbance (A) of the serum samples and standard were measured against the blank at wave length 500 nm by using cuvette of 1 cm light path of Sepctrophotometer.

** **Advantage of Blank Tube is to zero the instrument and eliminate all the effects of light (like refraction, diffraction, ..etc), measurement tube material and all other compounds in the solution except the target compound.**

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Calculations

$$\text{Level of cholesterol (mg/dL)} = \frac{\text{A (Sample)}}{\text{A (Standard)}} \times N$$

Where: N = Concentration of cholesterol in standard solution = 200 mg/dL.

Reference Range

Normal Cholesterol Level in Serum = < 200 mg/dL.

Borderline Risk Level = 200 – 240 mg/dL.

High Risk Level = > 240 mg/dL.