Cholesterol - EGD

(Trinder CHOD/POD End point)

Code : 11005/06/07/08 (5x10/2x50/2x100/5x100 ml)

(For the analyser/colorimetric estimation of cholesterol in Serum/Plasma)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST :

Cholesterol is both coming from food and synthesized by the human body, mainly in hepatic and intestinal cells. Cholesterol is a component of cells and organoids membranes. It is a metabolic precursor of bile acids, vitamin D and steroid hormones. Cholesterol, insoluble molecule, circulates associated with lipoproteins (HDL, LDL and VLDL).

Quantification of total cholesterol allows the detection of hypercholesterolemia, isolated or associated with hypertriglyceridemia. High cholesterol concentrations are associated with a high risk for vascular accident and apparition of atherosclerosis. The LDL/HDL ratio should be taken in to consideration for evaluating the risk of developing cardiovascular diseases.

PRINCIPLE :

Enzymatic determination of total cholesterol according to the following reactions.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ 4-Cholesterol-3-one + H_2O_2

$2H_2O_2$+Phenol+4-Aminoantipyrine $\xrightarrow{\text{peroxidase}}$ Quinoneimine+$4H_2O$

REAGENTS :

1. Cholesterol Reagent	5 x 10	2 x 50	2x100	5x100 ml
2. Cholesterol Standard	1	1	1	2x1 ml
3. HDL PPT Reagent	5	10	2x10	50 ml

The reagents are ready to use and usable to the expiration date when stored at 2-8°C & Protected from light, if contamination is avoided.

SAMPLE :

Serum.
Heparin or EDTA plasma from fasting patients.

EXPECTED RANGE :

Cholesterol
Normal : Up to 250 mg%
HDL Cholesterol : 30-63 mg% Male
35-75 mg% Female

LINEARITY :

Cholesterol kit is linear upto 700 mg%.

INSTRUCTIONS :

1. Avoid use of detergents for cleaning glassware. The presence of traces of detergent impurities interferes in the final color development.
2. All reagents are ready to use. Discard upon turbidity. Slight pink color (up to 0.15 Abs) does not effect the performance of the reagents.

DIRECTIONS FOR USE ON ANALYSERS :

Reaction Type	:	End point with std
Wave Length	:	500 nm (Green Filter)
Incubation temp	:	37°C
Incubation time	:	5 mins
Standard	:	200 mg%
Linearity	:	700 mg%
Unit	:	mg%

TOTAL CHOLESTEROL PROCEDURE :

Pipette in a clean dry test tube labelled as Blank (B) Standard(S), and Test (T)

Enzyme	B	S	T
Reagent	1ml	1ml	1ml
Deionized water	0.01ml	-	-
Standard	-	0.01ml	-
Serum/Plasma	-	-	0.01ml

Mix and read the optical density (OD) at 500nm against blank after a 5 minute incubation (37°C). The final colour is stable for at least 1 hour.

CALCULATION :

$$\text{Cholesterol Conc. in mg\%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 200 \text{ (Std. Conc)}$$

HDL CHOLESTEROL PROCEDURE :

Step I : precipitation

Serum	0.2 ml
HDL PPT Reagent	0.3 ml

Mix well and Stand at R.T. for 10 mins. Centrifuge at 3000 RPM for 10 mins.

Step II : Colour Development

Take 3 clean glass tubes lebelled as Blank (B), Standard (S) and Test (T)

	B	S	T
Enzyme Reagent (1)	1.0 ml	1.0 ml	1.0 ml
Cholesterol Std. (2)	-	0.01ml	-
Supernatant from Step 1	-	-	0.1ml
Distilled Water	0.1 ml	0.1ml	-

Incubate for 5 mins. at 37°C

Read the optical Density at 500 nm against Blank.

CALCULATION :

$$\text{HDL Conc.} = \frac{\text{Abs of Test}}{\text{Abs of Standard}} \times 50$$

NOTES :

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

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