Cholesterol (CHOD-POD: Enzymatic Photometric Method)

In-vitro Diagnostic reagent/kit for quantitative determination of Cholesterol in serum/plasma sample on Photometric System.

Reagent

Reagent : Enzyme solution
Standard: Cholesterol 200 mg/dL.

Principle

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase, H_2O_2 is liberated. The colorimetric indicator is quinoneimine is generated from 4 aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase (Trinder's reaction).

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acid}$$

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

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

Summary

Cholesterol is an integral part of cell membrane and a precursor for steroidal hormones and bile acids that are synthesized by cells and absorbed with food. Cholesterol is transported in blood via lipoproteins. There are different types of lipoproteins: High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) and Chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The increase or decrease in value of these Lipoproteins results in increase or decrease in Cholesterol concentration in one place. This results in risk such as coronary heart disease.

Storage Instructions and Reagent Stability

The reagent and standard is stable till the date of expiry, if stored at 2° - 8°C, protected from light and contamination is avoided.
Do not freeze the reagents.
Note: Measurement is not influenced by occasionally occurring color changes.

Components and Concentrations

Reagent: Pipes Buffer pH 7.0 100 mmol/L, Phenol 1gm/L, Cholesterol esterase (CHE) ≥ 150 U/L, Cholesterol Oxidase ≥ 100 U/L, Peroxidase (POD) ≥ 500 U/L, 4-Aminoantipyrine 0.5 mmol/L
Standard: Cholesterol 200 mg/dL.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagent and standard are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma Stability:
1 months at 2° – 8 °C, 3 months at -20 °C
Only freeze once!
Discard contaminated specimens.

Assay Procedure

Wavelength	505 (505 – 546) nm
Light path	10 mm
Temperature	37°C
Measurement	Against reagent Blank

	Blank	Sample/Standard/Calibrator
Sample	-	10 µL
Distilled water	-	-
Reagent	1000 µL	1000 µL
Mix, incubate for 5 min. at 37°C. Read absorbance against the reagent blank.		

Calculation:

With Standard or Calibrator

$$\text{Cholesterol (mg/dL)} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std. /Cal}} \times \text{Conc. of Std. /Cal (mg/dL)}$$

Quality Control

For internal quality normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

Warnings and Precautions

1. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
2. Wear suitable gloves and eye/face protection.
3. Always use safety pipettes to pull the reagents into a pipette.
4. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
5. The reagents contain sodium azide (0.95g/L) as preservative. Do not swallow. Avoid contact with skin and mucous membranes.
6. For professional use only!

Performance Characteristics

Measuring Range

Measuring range is 5 to 1000 mg/dL. When values exceed 1000 mg/dL, the samples should be diluted 1+1 NaCl solution (9g/L) and the result multiplied by 2.

Linearity/Limit of Maximum Detection

The maximum limit of detection of 1000 mg/dL.

Sensitivity/Limit of Detection

The lower limit of detection is 5 mg/dL.

Specificity/Interferences

No interference was observed by Ascorbic acid up to 5 mg/dL, Bilirubin up to 20 mg/dL, Triglycerides up to 2000mg/dL.

Precision

Intra assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	104.38	1.10	1.05
Sample 2	213.82	1.79	0.84
Sample 3	264.94	1.42	0.54

Inter assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	107.04	0.98	0.91
Sample 2	224.35	1.80	0.80
Sample 3	267.97	1.52	0.57

Method Comparison

A comparison of Precision Biomed Cholesterol (y) with a commercially available test (x) using 15 samples gave following results:
$y = 1.008x - 1.594$; $r^2 = 0.996$.

Reference Range

Desirable	≤ 200 mg/dL (5.2 mmol/L)
Borderline high risk	200 - 240 mg/dL(5.2 -6.2mmol/L)
High risk	> 240 mg/dL (> 6.2 mmol/L)

Note: It is recommended that each laboratory should establish its own reference range based on the patient population.

Quick Reference

Parameter	Cholesterol
Mode	End Point
Wavelength	505 (505 – 546) nm
Path length	10 mm
Standard conc.	200 mg/dL
Reagent volume	1000 µL
Sample volume	10 µL
Incubation time	5 min
Temperature	37°C
Blanking	Reagent blank
Normal range	< 200 mg/dL
Linearity	1000 mg/dL
Sensitivity	5 mg/dL

Pack Size :

Cat No.	Configuration	Pack
CHO00100	Reagent - 2 x 50mL Standard – 1 x 2mL	100mL
CHO00500	Reagent - 5 x 100mL Standard – 1 x 3mL	500mL
CHO01000	Reagent - 2 x 500mL Standard – 1 x 4mL	1000mL

Literature

1. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999.p.809-61
2. Eur Heart J 1998: 19 1434-503.
3. Handbook of lipoprotein testing. Washington: ACC Press, 1997:99-114.
4. Handbook of lipoprotein testing. Washington: AACC Press, 1997:25 - 48.

Version : CHO/00

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE
Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

Manufactured in India by :
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