

Cholesterol (CHOD-PAP: Enzymatic Photometric Method)



Order Information

Cat. No.
OMR1052

Kit Configuration
Reagent: 4 x 40 mL

Summary

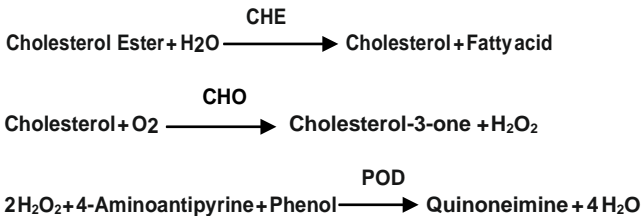
Cholesterol is an integral part of cell membrane and a precursor for steroidal hormones and bile acids that are synthesized by cells and absorbed with food. Cholesterol is transported in blood via lipoproteins. There are different types of lipoproteins: High Density Lipoproteins (HDL), Low Density Lipoproteins (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.

Method

CHOD-PAP: Enzymatic Photometric test

Principle

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase, H₂O₂ is liberated. The colorimetric indicator is quinoneimine is generated from 4 aminoantipyrine and phenol by H₂O₂ under the catalytic action of peroxidase (Trinder's reaction).



Reagent Storage Instructions and Stability

The reagent 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.

Composition

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.

Warnings and Precautions

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

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagent and the 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	546 nm
Light path	10 mm
Temperature	37°C
Measurement	Against reagent Blank

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

Calculation:

With Standard or Calibrator

ΔA Sample

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

Conversion Factor

$$\text{Cholesterol (mg/dL)} \times 0.02586 = \text{Cholesterol (mmol/L)}$$

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.

Performance Characteristics Measuring Range

Measuring range is 5 to 1000 mg/dL. When values exceed 1000 mg/dL, the samples should be diluted 1+4 NaCl solution (9g/L) and the result multiplied by 5.

Interferences

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

Sensitivity/Limit of Detection

The lower limit of detection is 5 mg/dL.

Linearity

The maximum limit of detection of 1000 mg/dL.

Precision

Intra assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	248.36	0.29	0.12
Sample 2	171.65	1.11	0.65

Inter assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	156.94	0.93	0.59
Sample 2	281.22	0.92	0.33

Method Comparison

A comparison of Nucleus Diagnosys Cholesterol (y) with a commercially available test (x) using 15 samples gave following results:

$$y = 1.003x + 0.217; r^2 = 0.999.$$

Reference Range

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

Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

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.

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