



CHOLESTEROL

Quantitative Determination of Cholesterol Enzymatic Colorimetric CHOD-POD Method

For professional in vitro diagnostic use only.

INTENDED USE

Quantitative determination of Cholesterol in Serum or Plasma.
Enzymatic Colorimetric CHOD-POD Method.

GENERALITIES

Cholesterol exists in the human blood as a free sterol and in an esterified form. The knowledge of the plasma level of lipids (cholesterol and triglycerides) together with lipoproteins of high and low density (HDL and LDL) aids in the detection of many conditions bound to metabolic disorders of high risk. The imbalance in the level of lipoproteins in plasma leads to hyper-lipoproteinemia, a group of disorders that affect lipid levels in serum, causing coronary heart disease (CHD) and atherosclerosis, conditions in which the cholesterol levels are important tools in their diagnosis and classification. Jaundice of the obstructive type usually is accompanied by an elevated total serum cholesterol with a normal ester fraction. Diabetes, hypothyroidism, and certain types of kidney disease are other disorders that may exhibit the same cholesterol disturbance. Low total cholesterol values with normal ester fractions are noted mainly in hyperthyroidism and malnutrition.

TEST PRINCIPLE

By enzymatic hydrolysis, the enzyme Cholesterol Esterase (CHE) frees Cholesterol from fatty acids. This fraction, together with the free part in the plasma, is oxidized by enzyme Cholesterol Oxidase (CHO) producing Hydrogen Peroxide (H_2O_2). The latter compound, by reaction catalyzed by the enzyme Peroxidase (POD) reacts with 4-amino-antipyrine to form the red quinone which can be measured photometrically (Trinder's reaction). The measurement of this Absorbance (optical density) is directly proportional to the cholesterol initially contained in the sample under examination. The use of a known calibrator makes quantitative analysis possible.

REAGENT COMPOSITION

Reagent

Good's buffer, pH 6.7	50 mmol/L
Phenol	5 mmol/L
4-Aminoantipyrine	0.3 mmol/L
Cholesterol Esterase (CHE)	≥ 300 U/L
Cholesterol Oxidase (CHOD)	≥ 200 U/L
Peroxidase (POD)	≥ 1200 U/L

Standard

Cholesterol	value on label
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STORAGE, PREPARATION AND SHELF LIFE

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents. Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge to avoid contamination, degradation from direct light and evaporation. The measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.150 at 505 nm.

SAMPLE COLLECTION AND PREPARATION

Serum, heparin plasma or EDTA plasma.

Avoid hemolyzed or lipemic samples. Separate the serum from the clot quickly.

Stability in serum/plasma: 3 days at 15° - 25 °C, 7 days at 2° - 8 °C, 3 months at -20 °C.

Discard contaminated samples. Freeze only once.

TEST PROCEDURE

Wavelength	: 505 nm
Light path	: 1 cm
Temperature	: 37 °C
Measurement:	: Against Reagent Blank
Assay type	: ENDPOINT

Assay:

	Blank	Assay
Reagent	1000 µL	1000 µL
Sample/ Standard/ Cal	/	10 µL
-Mix and incubate for 5 min at 37 °C.		
-Read the absorbance (A) of the samples and the standard against the reagent blank.		

CALCULATION

Serum/ Plasma:

$$\text{Cholesterol Concentration} = \frac{\text{Abs Sample} - \text{Abs blank}}{\Delta \text{Abs Std/Cal} - \text{Abs blank}} \times \text{Std/ Cal.}$$

$$\text{Conversion Factor: [mg/dL] x 0.026 = [mmol/L]}$$

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analyzed routinely with each group of unknown samples.

EXPECTED VALUES

Serum/plasma:

- Desirable: ≤ 200 mg/dL.
- Borderline high risk: 200 – 240 mg/dL.
- High risk: > 240 mg/dL.

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:

Low Level: Samples= 20; Average = 135; S.D. = 1.36; CV = 1.01%.

High Level: Samples = 20; Average = 218; S.D. = 3.10; CV = 1.41%.

ACCURACY:

A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

$$y = 1.005x - 0.281 \quad r = 0.999$$

SENSITIVITY: 7 mg/dL.

LINEARITY: 600 mg/dL.

PRECAUTIONS

R: contains PHENOL, 4-AMINOANTIPYRINE Harmful if swallowed, in contact with skin or inhaled. It causes severe skin burns and eye damage. Causes damage to organs.

in case of contact of reagents with the operator, you must apply the following first aid: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing, gloves and eye/face protection.

Note: Most of the detergents and water softening products used in the labs contain chelating agents. A defective rinsing will invalidate the procedure. Keep the glassware acid washed and thoroughly rinsed at all times.

INTERFERENCES

No interference was observed by Hemoglobin up to 500 mg/dL, Bilirubin up to 20 mg/dL, triglycerides up to 10 g/L.

LITERATURE

- Artiss JD, Zak B. Measurement of cholesterol concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997:99-114.
- Deeg R, Ziegenhorn J. Kinetic enzymatic method for automated determination of total cholesterol in serum. ClinChem 1983; 29:1798-802. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 22-3.
- Allain, C.C., Poon, L.S., Clau, C.S.G, Richmond, W and Fu, P.D. Clin. Chem. 20 : 470 (1974).
- Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

USED SYMBOLS

	In Vitro Diagnostic Medical Device
	Manufacturer
	Date of Manufacture
	Catalogue Number
	Batch Code
	Use by YYYY-MM (MM = end of month)
	Operator's Manual; Operating Instructions
	Keep away from Sunlight
	Keep away from Rain
	Temperature Limit
	Caution
	Do not use if Package is Damaged
	Do Not Re-Use
	Contains Sufficient for <N> Tests