Cholesterol

CHOD-POD. Enzymatic colorimetric

Quantitative determination of cholesterol

CE IVD

PACKAGING

Ref.: 101-0237	Cont.: 5 x 20 mL
Ref.: 101-0012	Cont.: 4 x 50 mL
Ref.: 101-0050	Cont.: 6 x 100 mL
Ref.: 101-0051	Cont.: 4 x 250 mL
Ref.: 101-0267	Cont.: 12 x 50 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

Cholesterol is a fat-like substance that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones. The determination of serum cholesterol is one of the important tools in the diagnosis an classification of lipemia. High blood cholesterol is one of the major risk factors for heart disease[5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

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

The intensity of the color formed is proportional to the cholesterol concentration in the sample[1,2].

REAGENTS

R 1 Buffer	PIPES pH 6.9	90 mmol/L
	Phenol	26 mmol/L
R 2 Enzymes	Cholesterol esterase (CHE)	300 U/L
	Cholesterol oxidase (CHOD)	300 U/L
	Peroxidase (POD)	1250 U/L
	4 – Aminophenazone (4-AP)	0.4 mmol/L
CHOLESTEROL CAL	Cholesterol aqueous primary standard 200 mg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Working reagent (WR): Dissolve the contents of one vial R 2 Enzymes in one bottle of R 1 Buffer.

Cap and mix gently to dissolve contents.

(WR) is stable: 4 months at 2-8º C or 40 days at 15-25º C.

Avoid direct sunlight.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm ≥ 0.1.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm (500-550).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1,2]: Stability of the sample for 7 days at 2-8º C or freezing at –20º C will keep samples stable for a few months.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CHOLESTEROL CAL: Proceed carefully with this product because due its nature it can get contamined easily.

LCF (Lipid Clearing Factor) is integrated in the reagent.

Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . 505 nm (500-550)
   Cuvette: . . . . . 1 cm light path
   Temperature . . . . . .37º C /15-25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard[(Note 1-2)] (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min. at 37º C or 10 min. at room temperature.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 60 minutes.

CALCULATIONS

$$\frac{(A)\,\text{Sample}}{(A)\,\text{Standard}} \times 200\ (\text{Standard conc.}) = \text{mg/dL cholesterol in the sample}$$

Conversion factor: mg/dL x 0.0258= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Risk evaluation[5,6]:

Less than 200 mg/dL	Normal
200 - 239 mg/dL	Borderline
240 mg/dL and above	High

These values are for orientation purpose; each laboratory should establish its own reference range.

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PERFORMANCE CHARACTERISTICS

Measuring range: From **detection limit** of 0.6 mg/dL to **linearity limit** of 600 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	90.1	305	90.4	301
SD	0.64	3.30	1.12	2.30
CV (%)	0.71	1.08	1.24	0.76

Sensitivity: 1 mg/dL = 0.002 A.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.995.
Regression equation: y= 1.004x – 0.931
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin up to 5 g/L and bilirubin up to 10 mg/dL, do not interfere[1,2].
A list of drugs and other interfering substances with cholesterol determination has been reported [3,4].

BIBLIOGRAPHY

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