Cholesterol-LQ

CHOD-POD. Liquid

Quantitative determination of cholesterol
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

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

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

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

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

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

CLINICAL SIGNIFICANCE

Cholesterol is a fat-like substance called a lipid 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 and 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.

REAGENTS

R	PIPES pH 6,9	90 mmol/L
	Phenol	26 mmol/L
	Cholesterol esterase (CHE)	1000 U/L
	Cholesterol oxidase (CHOD)	300 U/L
	Peroxidase (POD)	650 U/L
	4 – Aminophenazone (4-AP)	0,4 mmol/L
CHOLESTEROL CAL	Cholesterol aqueous primary standard 200 mg/dL. Contains Triton X-114 10-15%.	

PRECAUTIONS

CAL: H225- Highly flammable liquid and vapour. H318- Causes serious eye damage. H412- Harmful to aquatic life with long lasting effects.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

All the reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, 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 $\geq$ 0,26.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1,2]: Stability of the sample 7 days at 2-8ºC or freezing at –20ºC will keep samples stable for 3 months.

PROCEDURE

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

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard(Note 1,2,3,4) (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.
5. Read the absorbance (A) of the samples and standard, against the Blank. The colour is stable for at least 60 minutes.

CALCULATIONS

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

Conversion factor: mg/dL x 0,0258= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic .
If control values are found outside the defined range, check the instrument, reagent and calibration 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.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit 0,00 mg/dL to linearity limit 1000 mg/dL.
If the concentration is greater than linearity limit dilute 1/2 the sample with ClNa 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	99	201	96	197
SD	0,83	1,41	1,75	6,41
CV (%)	0,84	0,70	1,82	3,26

Sensitivity: 1 mg/dL = 0,0019 (A).
Accuracy: Results obtained using Audit Diagnostics reagents did not show systematic differences when compared with other commercial reagent.
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0,99549.
Regression equation: y=0,911x + 2,624.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed to hemoglobin up to 5 g/L and bilirubin up to 10 mg/dL[1,2].
A list of drugs and other interfering substances with cholesterol determination has been reported[3,4].

NOTES

1. CHOLESTEROL CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
2. LCF (Lipid Clearing Factor) is integrated in the reagent.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.
5. **Audit Diagnostics has instruction sheets for several automatic analyzers.**

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