LIQUID CHOLESTEROL (CHOD/POD method)

For the determination of cholesterol in serum or plasma

IVD *For in -vitro diagnostic use only*

Store at 2-8°C

INTENDED USE

For the measurement of cholesterol concentration in human serum or plasma.

INTRODUCTION

Cholesterol is one of the lipids found in the blood stream. A high level of cholesterol in the blood — hypercholesterolemia — is a major risk factor for coronary heart disease, which leads to heart attack.

METHODOLOGY: CHOD/POD method.

PRINCIPLE OF THE METHOD

The enzyme cholesterol esterase is used to hydrolyze the cholesterol esters present in the serum to free cholesterol and free fatty acids. The enzyme cholesterol oxidase in the presence of oxygen to oxidizes the cholesterol to cholest-4-en-3one and hydrogen peroxide. Hydrogen peroxide oxidizes phenol and 4-aminoantipyrine to produce red color that can be measured spectrophotometrically.

Cholesterol esters + H_2O	CHE →	cholesterol +free fatty acids
Cholesterol + O_2	CHOD →	cholest-4-en-3-one + H_2O_2
$2H_2O_2$ + Phenol + 4-AA	POD →	Quinoneimine dye + 4H_2O

The intensity of the color formed is proportional to the cholesterol concentration in the serum.

REAGENTS COMPOSITION

R	PIPES PH 6.8	90mmol/L
	Phenol	26mmol/L
	4-Aminophenazone(4-AA) 0.4mmol/L	
	Cholesterol esterase(CHE)	1000U/L
	Cholesterol oxidase(CHOD)	300U/L
	Peroxidase (POD)	650U/L
Cholesterol STD	Cholesterol aqueous primary standard 200mg/dl	

PREPARATION

- Reagent and standard provided 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 2-8°C, protected from light and contaminations during their use.
- Do not use reagents after the expiration date.
- SIGNS OF REAGENT DETERIORATION
  - Presence of particles and turbidity.
  - Blank absorbance against water at 505 nm ≥ 0.26

EQUIPMENTS NEEDED BUT NOT PROVIDED

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

COLLECTING AND HANDLING OF SPECIMENS

Use serum, or plasma preserved in EDTA

Determination of lipid constituent in plasma or serum are normally done on blood drawn from patients fasting for 12 to 16 hours. Stability of the sample is 7 days at 2-8°C. Freezing at -20ºC will keep samples stable for 3 months. Freezing at -60°C provides the longest stable storage and may allow for Reproducible results even after a year or more.

ASSAY PARAMETERS

Reaction	End point	Sample Vol.	0.01ml
Wavelength	505 nm	Reagent Vol.	1.0ml
Zero Settings	Reagent blank	Standard	200mg/dl
Incub. Temp.	37°C/R.T	linearity	Up to 600 mg/dl
Incub. Time	5 min/10		
	min		
Reac. Slope	increasing		
Units	mg/dl		

ASSAY PROCEDURE

1. Wavelength…… 505 nm (500-550)
2. Cuvette……1cm.light path
3. Temperature……37ºC/25ºC
4. Adjust the instrument to zero with distilled water.
5. Pipette into clean dry test tubes labeled as Blank (B), Standard(S), and Test (T):

	B	S	T
Reagent(ml)	1.0	1.0	1.0
Standard(µL)	-	10	-
Sample (µL)	-	-	10

6. Mix well and incubate at 37C for 5min or at R.T. (25C) for 10min.
7. Measure the absorbance of the standard and test sample against blank.
8. After incubation the color is stable for at least 60 min.

CALCULATIONS

$$\text{Cholesterol (mg/dl)} = \frac{\text{(A) Sample}}{\text{(A)STD}} \times 200 \text{ mg/dl (STD Conc.)}$$

Conversion factor: mg/dL x 0.02586= mmol/L.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each run includes assayed normal and abnormal controls. If control values are found outside the defined range, check the instrument calibration, and reagent for problems.

REFERENCE VALUES

Serum or plasma:

Classification	Total cholesterol (mg/dl)
Desirable	<200
Borderline to high risk	200-239
High risk	≥ 240

These values are for guidance purpose; each laboratory should establish its own reference range, according to its own geographic area.

PERFORMANCE CHARACTERISTICS

Measuring range (Linearity):

The assay is linear between 10 mg/dl and 600 mg/dl. If the results obtained were greater than linearity limit, dilute the sample to 1/2 with Nacl 9g/L and multiply the result by 2.

***Sensitivity*:**

1 mg/dl = 0.0016 (A)

Accuracy:

Results obtained using reagent compared well with other commercial reagents.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean(mg/dl)	84.256	199.395	99.58	217.02
STD	1.46	6.46	1.12	6.21
C.V%	1.73%	3.2%	1.12%	2.89%

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed to hemoglobin up to 5 g/L and up to 10mg/dl. A list of drugs and other interfering substances with cholesterol determination has been reported by Young et.al.

NOTES

- LCF (Lipid Clearing Factor) is integrated in the reagent, that clears the
- turbidity caused by lipemic sample and thus avoids overestimation of
- Cholesterol.
- 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.

REFERENCES

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REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone number		