CHOLESTEROL (TOTAL)

CHOD-PAP Method

Intended Use:
Total Cholesterol (Total) Test reagent/kit is a medical device intended for estimation of Cholesterol in serum or plasma.

Clinical Significance
Cholesterol is a fat like substance that is found in all body cell. The liver makes all 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 factor of heart disease[5,6].
Clinical Diagnostic should not be made on a single test result. It should integrate clinical and other laboratory data.

Principle

Cholesterol esters in Serum are hydrolysed by cholesterol esterase (CHE). The free cholesterol produced is oxidized by cholesterol oxidase (Co) to form Cholest 4en- 3-onewithsimultaneous production of hydrogen peroxide (H_2O_2) which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield a red chromophore.

Cholesterol ester + H_2O $\xrightarrow{CHE}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow{CHO}$ Cholesterol-3-one + H_2O_2

2 H_2O_2 + 4-AP + Phenol $\xrightarrow{POD}$ Quinoneimine + 4 H_2O

Reagent Composition

R1 Cholesterol Reagent	Phosphate Buffer- 100 mmol/L CHOD – 900U CE – 1200U POD – 2000U Activators and stabilizers
Std Conc	200mg/dl

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage

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 contamination prevented during their use.

Do not use reagent beyond the expiry date.

Discard if signs of deterioration appear:
- Presence of particles and turbidity
- Blank Absorbance (A) at 505 nm >0.160

Standard reading is abnormally low.

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

Sample and Stability

Serum or plasma

Stability of the sample for 7 days at
2 - 8°C or freezing at - 20°C will keep the sample for 3 months

Assay Procedure
Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T)

Addition Sequence	Blank	Std	Test
R1 Cholesterol (Total) Reagent	1000µl	1000µl	1000µl
Standard (S)	-	10µl	-
sample	-	-	10µl

Mix, incubate 5 min. at 37 °C or 10 min. at Room Temperature (25 -30°C).
Read absorbance against the blank at 505nm or green filter (500-546nm).

Calculations

$$\text{Cholesterol (Total) in mg/dl} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 200$$

Conversion Factor: mg/dL X 0.0258 = mmol/L

Quality Control
Commercially available normal and pathological control sera are recommended to monitor the performance of the procedure.
If the controls are found outside the defined range, check the instrument, reagents and calibrator for problems.

Linearity
Up to 1000 mg/dl, under the described assay conditions. When values exceed this range the samples should be diluted appropriately with NaCl solution (9 g/l) and the result multiplied by the dilution factor.

Reference Values
Serum / Plasma : 130 – 250 mg/dl

Risk Evaluation[5,6] :

Less than200mg/dL	Normal/Recommended
200 –240mg/dL	Borderline
>240mg/dL	High Risk

These values are for orientation purpose
Each laboratory should establish its own reference range

General System Parameters

Mode	End Point
Reaction	Increasing
Wavelength	505 nm (500-546)
Blank with	Reagent
Sample Volume	10µl
Reagent Volume	1000µl
Std Conc.	200mg/dl
Incub. Temp.	37°C
Incub. Time	5 min.
Delay Time	5 sec
Normal Range	130 – 250mg/dl
Linearity	Up to 1000mg/dl
Unit	mg/dl

Performance Characteristics

Measuring range: From detection limit of 0.113 mg/dl to linearity limit of 1000 mg/dl.

If the concentration is 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)	99.789	185.309	96.346	184.962
SD	1.213	1.405	4.196	12.773
CV (%)	1.216	0.758	4.355	6.906

Sensitivity: 1 mg/dl = 0.0015 A.

Accuracy: Results obtained using this reagents did not show systematic differences when compared with other commercialreagents.

The results obtained using 50 samples were the following: Correlation coefficient (r): 0.997

Regression equation: y=0.9797x + 2.2803

The results of the performance characteristics depend on the analyzerused.

Notes

1. Cholesterol Calibrator: proceed carefully with this product; due to its nature it can get contaminated.

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. Use clean disposable pipette tips for its dispensation.

References

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For *in vitro* Diagnostic use only.