



# CHOLESTEROL

(CHOD-POD Method)  
Liquid Reagent

## INTENDED USE:

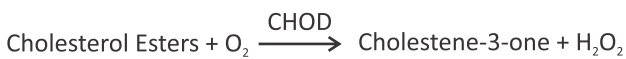
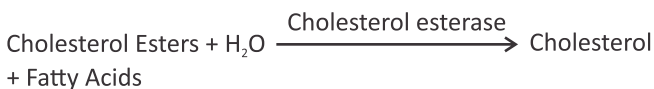
This reagent kit is used for *in-vitro* quantitative determination of Total Cholesterol in human serum and plasma.

## TEST PRINCIPLE:

Cholesterol methodologies have been critically reviewed by Tonks(1) and more recently by Zak(2). The enzymatic method described below, and used in this assay, is a modification of that described in 1974 by Allain et.al. (3) and Roschlau et.al (4). The use of enzymes to assay cholesterol has been studied by many investigators (5,6,7) This reagent is based on the formulation of Allain, et. al.(3) and the modification of Roschlau (4) with further improvements to render the reagent stable in solution.

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide combines with phenol and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which is quantitated at 500 nm.

## REACTION :



## KIT CONTENTS:

**Reagent 1:** Cholesterol Enzyme Reagent

**Reagent 2 :** Cholesterol Standard (200 mg/dl)

**Product Insert** : 01 No.

## PREPARATION OF THE WORKING REAGENT:

All the reagents are ready to use.

## STORAGE AND STABILITY:



All the reagents should be stored in 2-8°C and are stable till the expiry date mentioned in the labels.

## SPECIMEN COLLECTION AND STORAGE:

Unhemolysed serum (fasting) is recommended. Heparinised plasma may be used. Cholesterol in serum is stable for seven days at room temperature and six months when frozen and properly protected against evaporation.

## PRECAUTIONS: ⚠

- Storage conditions as mentioned on the kit to be adhered.
- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.

- Before the assay bring all the reagents to room temperature.
- After use store the kit contents immediately as 2-8°C.
- Avoid contamination of the reagent during assay process.
- Use clean glassware free from dust or debris.

## PROCEDURE (Automated): ⓘ

Refer to specific instrument application instructions.

## TEST PROCEDURE (Manual): ⓘ

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

Pipette into Test Tube	Blank	Standard	Test
Cholesterol Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix well and incubate for 5 minutes at 37°C.

Read absorbance of Standard ( $A_s$ ), and Test ( $A_T$ ) against Blank ( $A_b$ ) at 505 nm or with green filter (500 - 540 nm).

## CALCULATIONS:

Cholesterol Conc. in mg/dl =

$$\frac{\text{Abs of } A_T - A_b}{\text{Abs of } A_s - A_b} \times 200 \text{ (Conc. of Standard)}$$

## NORMAL VALUES\*:

Serum/Plasma : 130 - 250 mg/dl

\*It is recommended that each laboratory should establish its own normal range representing its patient population.

## PERFORMANCE:

- Linearity:** 1000 mg/dl
- Comparison:**  $r = 0.99$   
 $y = 1.1x + 7.5$
- Precision:**

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
<b>Low</b>	85.0	1.4	1.4	85.0	1.5	2.0
<b>High</b>	195.0	1.4	0.4	285.0	5.5	2.1

**4. Specificity:** No interference, enzymes are specific.

## CLINICAL SIGNIFICANCE:

Increased levels of cholesterol may be found in coronary artery disease, uncontrolled diabetes mellitus, hypothyroidism, nephrotic syndrome and hepatic malfunction. Cholesterol level

may be low in acute hepatitis, malnutrition, anaemia, hyperthyroidism and Gaucher's disease. Normal Cholesterol levels can be altered by age, stress, pregnancy and hormonal imbalance.

#### **AUTOMATED APPLICATIONS:**

Cholesterol Liquid reagents can be used with Hitachi 700 series, RA 50, 1000 XT, Express 550, Synchron CX4, LISA 200, BTR 810/820/830, Erbachem-5, Ranlab etc. Application sheets for use on specific semiautomatic / batch analysers are available on request.

Input parameters for semiauto / auto analyzers are given below:

<b>INPUT PARAMETERS</b>	<b>VALUES</b>
Type of reaction	End point
Wavelength	505 nm.
Incubation time	5 minutes
Standard concentration	200
Units	mg/dl
Temperature	37°C
Upper Normal value	250 mg/dl
Lower Normal value	130 mg/dl
Linearity	1000 mg/dl
Reagent volume	1.0 ml
Sample/Standard volume	10 µl

#### **QUALITY CONTROL:**

For accuracy, it is necessary to run known serum controls with each assay.

#### **REFERENCES:**

1. Zak, B., Cholesterol Methodologies: A Review, Clin. Chem. 23,1201 (1977).
2. Allain, C.C., Poon, L., Chan, S.G., Richmond, W., Fu, P., Enzymatic Determination of Total Serum Cholesterol, Clin. Chem. 20, 470 (1974).
3. Pesce, M.A., Bodourian, S.H., Interference with the Enzymatic Measurement of Cholesterol in Serum by Use of Five Reagent Kits, Clin. Chem. 23, 757760 (1977).
4. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Washington, Third Edition, 1990.