CHOLESTEROL

(CHOD-PAP, Enzymatic)

ENZOPAK

Last update 02-2026

Ref. CC2-CLE.005, 5x25 ml
CC2-CLE.05U, 5x60 ml
CC2-CLE.05V, 20X60 ml

INTENDED USE

Reagent kit for quantitative estimation of Cholesterol in serum or Plasma.

PRODUCT HIGHLIGHTS

Reagents made to customer needs

- Pack sizes offered suitable to every laboratory
- Linearity upto 1000 mg/dL.
- Stabilized aqueous standard
- Low blank all throughout shelf life.

INTRODUCTION

In 1885, the first method of cholesterol analysis was reported by Lieberman and shortly thereafter by Burchard. In the reaction, sulphuric acid, in highly acidic dry medium, acts on cholesterol and cholesterol esters to form blue green coloured compounds. In 1958, Abell introduced a method using an alkaline hydrolysis of esters and Lieberman Burchard reaction. Several methods with or without hydrolysis using Lieberman Burchard reaction were developed. However, these methods used corrosive acids, involved extraction, protein precipitation and were cumbersome and time consuming.

The first step towards fully enzymatic procedure was the use of cholesterol oxidase after specimen hydrolysis as described by Flegg, Richmond and Roeschlau.

Cholesterol is formulated on the modified method described by Allain and Roeschlau, et al in 1974.

DIAGNOSTIC SIGNIFICANCE

Age, sex, hormonal imbalance, stress and pregnancy affect normal cholesterol levels. However, high levels of cholesterol are noted in coronary artery disease, diabetes mellitus, hypothyroidism, nephrotic syndrome and cirrhosis of liver. On the other hand, lower cholesterol levels may be noted in anemias, malnutrition, severe hepatitis, Gaucher's disease and hyperthyroidism.

PRINCIPLE

The cholesterol esters are hydrolysed to free cholesterol by cholesterol esterase (CE). The free cholesterol is then oxidized by cholesterol oxidase (CO) to cholesten-4-en-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and phenolic compound in the presence of peroxidase to yield a coloured complex, which is read at 505 nm (500-540 nm, GREEN Filter).

$$\text{Cholesterol esters} \xrightarrow{CE} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholesten-4-en-3-one} + H_2O_2$$

$$\left.\begin{array}{c} H_2O_2 + 4AAP \\ + \\ \text{Phenolic Compound} \end{array}\right\} \xrightarrow{\text{Peroxidase}} \left\{\begin{array}{c} \text{Quinoneimine dye} \\ + \\ 4H_2O \end{array}\right.$$

The intensity of colour produced is directly proportional to the concentration of total cholesterol in the sample.

PRESENTATION

All reagents to be stored at 2-8°C	No. of Bottles		
	5 x 25 ml	5 x 60 ml	20 x 60 ml
• 1 Cholesterol (Enz-Chromogen)	5	5	20
• 2 Cholesterol (Diluent)	1	5	20
• Cholesterol Standard (200 mg/dL)	1	1	4

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Cholesterol Oxidase	≥ 500 U/L
• Cholesterol Esterase	≥ 600 U/L
• Peroxidase	≥ 6000 U/L
• 4-Amino Antipyrine	0.5 mmol/L
• Buffer	100 mmol/L
• Detergent	15 mmol/L
• Phenol	20 mmol/L
• Surfactant	20 mmol/L
• pH 7.00±0.1 at 25^{0}C	

Cholesterol Standard (200 mg/dl)

Also contains non-reactive fillers and Stabilizers.

PRECAUTION

CHOLESTEROL is for *IN-VITRO* diagnostic use only.

Reagent contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT

For 5x25 ml

Dissolve the contents of one vial of 1 Cholesterol (Powder) with 25 ml of 2 Cholesterol (Diluent). Mix well to dissolve the contents. Wait for 10 minutes before use.

For 5x60 ml & 20x60 ml

Carefully transfer the content of 1 Cholesterol (Powder) into the bottle containing 60 ml of 2 Cholesterol diluent Mix to dissolve the contents. Wait for 10 minutes before use.

REAGENT STORAGE AND STABILITY

All reagents included in the kit are stable at 2-8^{0}C until the expiry date stated on the label. The working reagent is stable for 6 months at 2-8^{0}C. On storage, there will be slight rise in the reagent blank but it will not affect the test results.

SPECIMEN COLLECTION

Fresh, clear serum under fasting condition is the specimen of choice. However, plasma collected from blood using heparin as an anticoagulant under similar conditions may also be used.

Note: A special surfactant, Lipid Clearing Factor (LCF) is added to the reagent to solubilise the lipemic sera, which adds to the accuracy of results.

REACTION PARAMETERS

- Type of Reaction : End Point
- Wavelength : 505 nm (500-540 nm)
- Flowcell Temperature : 37^{0}C
- Incubation : 10 min. at 37^{0}C
- Std. Concentration : 200 mg/dl
- Sample Volume : 10 µl (0.01 ml)
- Reagent Volume : 1.0 ml.
- Zero setting with : Reagent Blank
- Light Path : 1.0 cm.

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TEST PROCEDURE

Pipette Into Test Tubes	Blank	Standard	Test
Working Reagent (ml)	1.0	1.0	1.0
Standard (ml)	-	0.01	-
Sample (ml)	-	-	0.01

Mix well and incubate for 10 minutes at 37^0C. Read absorbance of test and standard at 505 nm (500-540 nm) or with Green filter against reagent blank.

STABILITY OF FINAL REACTION MIXTURE

The colour of the final reaction mixture is stable for 30 minutes when kept in cool dark place.

TEST RESULTS

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Std.}}{\text{Absorbance of Test}} \times 200$$

LIMITATIONS FOR INTERFERENCE

As per studies carried out for interference. Following results were obtained.

(a) No Interference from Hemoglobin upto 375 mg/dl.
(b) No Interference from free Bilirubin upto 7.5 mg/dl.
(c) No Interference from Lipemic (Measured as Triglycerides) upto 1000 mg/dl.

NORMAL VALUES

140 to 250 mg/dl.

LINEARITY

This procedure is linear upto 1000 mg/dl. For sample values higher than 1000 mg/dl, dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain test results.

NOTE

For Laboratories using instruments with cuvette capacity less than 1.0 ml, sample and working reagent volumes should be proportionately decreased.

REFERENCES

1. ROESCHLAU, P., et al: Methods of Enzymatic Analysis. 2nd English Edition, H.U. Bergmeyer, ed. Academic Press, New York (1974), 1980.
2. FLEGG. H.M. Ann. Clin. Biochem., 10:79 (1973).

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