Spectrum For Diagnostic Industries

Cholesterol
CHOD-PAP

IVD

REF.	Pack size
110 01 050	(1 x 50 ml) 50 tests
110 02 030	(2 x 30 ml) 60 tests
110 05 030	(5 x 30 ml) 150 tests

Intended Use

Cholesterol reagent is intended for in-vitro quantitative and diagnostic determination of cholesterol in human serum on both manual and automated systems.

Introduction

Measurement of serum cholesterol levels is important as an indicator of liver function, intestinal absorption, biliary function and in the diagnosis and classification of hyperlipoproteinemias.
Elevated cholesterol levels may occur with hypothyroidism,diabetes and nephrotic syndrome. Elevated serum cholesterol levels correlate well with the incidence of coronary artery diseases. Stress, age, gender, hormonal balance and pregnancy affect normal cholesterol levels. Depressed levels are associated with hyperthyroidism and severe liver diseases.

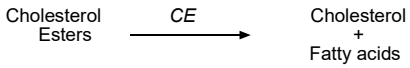
Method

CHOD-PAP-enzymatic colorimetric method.

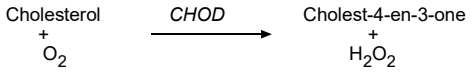
Principle

The series of the reactions involved in the assay system is as follows:

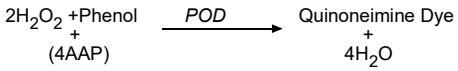
1. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase (CE) to cholesterol and free fatty acids.



2. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CO) to cholest-4-en-3-one and hydrogen peroxide.



3. The hydrogen peroxide combines with phenol and 4-amino-antipyrine (4AAP) in the presence of peroxidase (POD) to form a chromophore (quinoneimine dye) which may be quantitated at 500 – 550 nm. For bichromatic analyzers the blank wavelength should be set to 600 or 650 nm.



Reagents

Reagent

Pipes Buffer	50 mmol/L
Phenol	30 mmol/L
Sodium cholate	5.0 mmol/L
CE	>250 U/L
CHOD	>500 U/L
POD	>2.0 KU/L
4AAP	1.0 mmol/L
Azide	8.0 mmol/L

Standard

200 mg/dL	5.17 mmol/L

Reagents preparation, storage and stability

Cholesterol reagent is supplied ready-to-use and stable up till the expiration date labeled on the bottles.Once opened, the reagent and the standard are stable for 3 months at the specified temperature.

Deterioration

The reagent is normally clear or pale pink. Do not use liquizyme cholesterol reagent if it is turbid or if the absorbance is greater than 0.15 at 546 nm.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
Reagent contains sodium azide which may react with copper or lead plumbing.

Specimen collection and preservation

It is recommended that prior to sample collection, patients should be following their usual diet and be in their usual state of health. Patients who are actually ill, losing weight, pregnant or have had a myocardial infarction in the previous 3 months should be resch-eduled. Both fasting and non fasting samples can be used. Non haemolysed serum or plasma can be stored at 4 ^{o}C up to 7 days prior to analysis, 5-7 days at 20-25^{o}C, stable for 3 months at -20 ^{o}C, and at -70 ^{o}C for several months. The only acceptable anticoaglulant is heparin.

Procedure

Wavelength	546 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 100
Temperature	15 – 25 ^{o}C or 37 ^{o}C
Zero adjustment	Reagent blank
Incubation time	5 minutes at 37 ^{o}C or 10 minutes at 15 – 25 ^{o}C
Reagent Blank Limits	Low 0.00 AU High 0.15 AU
Sensitivity	5 mg/dL (0.13mmol/L)
Linearity	750 mg/dL (19.5 mmol/L)

	Reagent blank	Standard	Specimen
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard	--------	10 µl	-------
Specimen	--------	-------	10 µl

Mix and incubate for 5 minutes at 37 ^{o}C or 10 minutes at 15 – 25 ^{o}C. Measure absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank within 30 min.

Calculation

$$\text{Serum cholesterol conc. (mg/dL)} = \frac{(A_{specimen})}{(A_{standard})} \times 200$$

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run.

Interference

Haemolysis
No significant interference up to a level of 500 mg/dL.

Icterus
No interference from free bilirubin up to a level of 15 mg /dL (260 mmol/L) and conjugated bilirubin up to a level of 7 mg/dL (116 mmol/L).

Lipemia
No significant interference up to 1.7 AU.

Drugs
Of the drugs tested in vitro, Methyldopa causes artificially low total cholesterol values at the tested drug Level.

Others
Physiological ascorbic acid concentration does not interfere with the test. Ascorbic Acid levels higher than 425 mmol/L (7.5 mg/dL) decrease the apparent total cholesterol concentration significantly.

Expected Values

The following guidelines may be used for clinical interpretation:

Risk classification	Total cholesterol	
Desirable	<200 mg/dL	<5.2 mmol/L
Borderline high	200-239 mg/dL	5.2-6.2 mmol/L
High	>240 mg/dL	>6.2 mmol/L

Performance Characteristics

Precision
Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	149.8	252
SD	1.69	1.91
CV%	1.13	0.76

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	157	259
SD	1.77	2.12
CV%	1.23	0.97

Method Comparison
A comparison between Cholesterol reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.988 was obtained.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 5 mg/dL (0.13 mmol/L).

Linearity
The reaction is linear up to a cholesterol concentration of 750 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Flegg HM : Ann Clin Biochem 1963 .
2. NCEP expert panel, Arch Intern Med 1988.
3. Young DS .et al. Clin Chem.
4. Trinder, P, Ann. Clin. Biochem.

SYMBOLS IN PRODUCT LABELLING

IVD — For in-vitro diagnostic use
LOT — Batch Code/Lot number
REF — Catalogue Number
Consult instructions for use
Temperature Limitation
Use by/Expiration Date
CAUTION. Consult instructions for use
Manufactured by

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