todylaboratories®

Clinical Chemistry

CE IVD REF C23CC1057

Cholesterol CHOD-PAP · Liquid Stable
Instructions For Use

For in-vitro diagnostic use only.
Store at 2-8°C.

KIT CONTENTS

R1 Chol Reagent	2 x 60ml
R4 Standard	1 x 5 ml

TEST PRINCIPLE

Cholesterol is present in serum as cholesterol esters and free cholesterol. The Cholesterol esters present in serum are hydrolysed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine present to form the red quinoneimine dye. The intensity of the dye formed is directly proportional to the level of cholesterol present in the sample.

CLINICAL SIGNIFICANCE

Elevated levels of cholesterol are primarily considered as an indication of increased risk of cardiovascular disease and should be taken into consideration combined with the overall lipid profile.

REAGENT CONCENTRATION

Chol Reagent	Pipes Buffer	50 mmol/l
	Cholesterol Oxidase	> 100 U/l
	Cholesterol Esterase	> 150 U/l
	4-aminoantipyrine	0.3 mmol/l
	Peroxidase	> 800 U/l
	Phenol	6.0 mmol/l
Standard	Cholesterol	200 mg/dl

REAGENT HANDLING AND PREPARATION

The reagent is supplied ready to use. It is stable for up to the expiry date when kept at 2-8°C and protected from light. The reagent may develop a slight pink colouration. This will not affect performance providing the OD remains <0.300 when measured against a water blank at 505nm. Reagent is stable on-board for 28 days.

SAMPLE

Serum/Heparinised Plasma
EDTA plasma may also be used but tends to give lower results.

SPECIMEN

Do not use citrate, oxalate or fluoride.
Fasting and non-fasting samples can be used.
Centrifuge samples containing precipitate before performing assay.

STABILITY

5-7 days at +2 to +8°C
3 months at -20°C

MANUAL PROCEDURE

Wavelength	Temperature	Cuvette	Measurement
500 nm, Hg 546nm	20-25°C, 37°C	1 cm light path	Against Reagent Blank

Pipette into test tubes as follows:		
	Blank	**Standard / Sample**
DDH_2O	10 µl	-
Standard/Sample	-	10 µl
Chol Reagent (R1)	1000 µl	1000 µl
A serum based calibrator or an aqueous cholesterol standard can be used to calibrate this assay. Mix well and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C, then read the absorbance of the sample or standard against the reagent blank. The endpoint is stable for 60 minutes.		

CALCULATION

$$\text{Cholesterol conc in sample} = \frac{\Delta\text{abs Sample}}{\Delta\text{abs standard}} \times \text{conc. of standard}$$

LINEARITY

The test is linear up to a cholesterol concentration of 19 mmol/l (735 mg/dl). Dilute samples above this concentration with 0.9% NaCl and reassay, multiplying the result by the dilution factor.

SENSITIVITY

Cholesterol levels of 0.30 mmol/l (12 mg/dl) can be measured accurately by this method.

PRECISION

Intra Assay – Within run		
Cholesterol conc (mmol/l)	n	CV%
4.50	20	2.1 %
5.18	20	1.7 %

Inter Assay – Between Run		
Cholesterol conc (mmol/l)	n	CV%
4.50	10	3.9 %
5.18	10	3.5 %

These characteristics were determined using an AU600 analyser. Results will vary depending on the system in use.

METHOD COMPARISON

A comparison of Cholesterol (y) with a commercial obtainable assay (x) gave the following result:
y = 0.948 x +3.92; r = 0.99

LIMITATIONS - INTERFERENCE

Haemoglobin values up to 200mg/dl & bilirubin values up to 5mg/dl do not interfere with the test.

NORMAL VALUES

Cholesterol Level	Clinical Interpretation
< 5.2 mmol/l (200 mg/dl)	Normal
5.2-6.2 mmol/l (200-239 mg/dl)	Borderline Risk
> 6.2 mmol/l (240mg/dl)	High Cholesterol
These values are intended only as a guideline. Cholesterol levels can vary seasonally, according to geographic location and with time of sampling. At least 2 measurements should be made on separate occasions and the results should be taken in conjunction with other clinical and laboratory information.	

USE ON AUTOMATED ANALYSERS

This reagent is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.
For automated use we recommend a serum based calibrator to eliminate any matrix bias which may be observed with the aqueous standard.

QUALITY CONTROL

It is recommended that a laboratory uses normal and elevated reference control sera to verify the performance of the procedure, both performance of the reagent and any instrumentation employed in the determination. Results obtained should fall within the specified ranges.
Normal Bovine Assayed Control
Elevated Bovine Assayed Control
Normal Human Assayed Control
Elevated Human Assayed Control
QC materials of human source have been tested at donor level for HbsAg Antigen, HIV1&2 antibodies and HCV antibody and found to be negative. However no test can offer complete assurance to the absence of infectious diseases so all material should be handled and disposed of as if it is potentially infectious.
If results fall outside the acceptable range appropriate action as determined by the laboratory's internal quality procedures should be taken.
Some common reasons for incorrect results can be:
1. Wavelength used for the determination
2. Light source
3. Temperature
4. Cleanliness, e.g of cuvettes used in measurements
5. Bacterial contamination of reagent
6. Reagent expiry
7. Calibration frequency

HEALTH & SAFETY

This kit is designed for use by suitably qualified laboratory personnel only. Exercise the normal precautions required for the handling of laboratory reagents. Do not ingest the material. Dispose of material according to local guidelines.

REFERENCES

1. Tietz NW *Clinical Guide to Laboratory Test* 3rd Edition
2. Richmond N Clin. Chem 1973; 19: 1350-1356
3. Recommendations for improving Cholesterol Measurement; A report from the Lab, Standardization Panel of the National Cholesterol Education Program NIH Publication No 90-2564, Feb 1990
4. Trinder P *Annals of Clinical Biochemistry*. 1969

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SYMBOL INDEX

IVD For In Vitro Diagnostics Use Only	Expiry Date (Year/Month)
LOT Lot Number	Warning, Read Enclosed Documents
REF Catalogue Number	Instructions For Use
Storage Temperature	Manufactured By

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