CliniChem
H-1117 Budapest,
Budafoki street 111-113
Tel.: +36-1-205-3617
Tel./Fax: +36-1-205-3616

CHOLESTEROL PAP

FREEZE DRIED

Cat. No.:	40131	40132
	9x50 ml	90x50 ml

Reagent kit for the quantitative determination of total cholesterol concentration in serum. Enzymatic colorimetric method (PAP).

The biosynthesis of Cholesterol predominantly takes place in the liver and in intestinal mucosa, but almost all cells synthetize it. It is a constituent of many membranes, it is also essential in the synthesis of bile acids and steroid hormones. It circulates in blood as cholesterol ester bound to beta lipoproteins. The measurement of the level of Cholesterol as well as Triglycerides and Lipoproteins is important in examining the metabolism of lipids. Changes in the level of Cholesterol mainly reflect disorders of liver function. Cholesterol level is increased in obstructive jaundice, diabetes mellitus and hypothyroidism. The level is decreased in some cases of hyperthyroidism and certain forms of anaemia. Identification of the different density fractions (HDL,LDL,VLDL) as well as total Cholesterol plays a role in the diagnosis.

Principle

The Cholesterol esters of the sample are hydrolyzed by Cholesterol esterhydrolase (ChEH). 4-Cholesten-3-one and H_2O_2 are then formed from the released free Cholesterol by Cholesterol oxidase (ChOD)A measurable Red quinonimine derivative which absorbance light at 505 nm is formed from Hydrogenperoxide (H_2O_2) and 4-Aminoantipyrine in the presence of Phenol and peroxidase (POD).

$$Cholesterol\ ester + H_2O \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} Cholest\text{-}4\text{-}en\text{-}3\text{-}one + H_2O_2$$

$$2H_2O_2 + Phenol + 4\text{-}Aminoantipyrine \xrightarrow{Peroxidase} Red\ quinone + 4H_2O$$

Reference values

Serum cholesterol: 2.8-5.2 mmol/l (109-202 mg/dl)

It is recommended that each laboratory should assign its own normal range.

Reagents

1. Reagent (R1)

Pipes buffer, pH=6.90	90 mmol/l
Phenol	26 mmol/l

2. Reagent (R2)

4-Aminoantipyrine	0.4 mmol/l
Cholesterol esterase	300 U/l
Cholesterol oxidase	300 U/l
Peroxidase	1250 U/l

Samples

Serum free of haemolysis.

PROCEDURE

Preparation and stability of working reagent

Dissolve one vial of reagent 2 (R2) in the reagent 1 (R1).

Stability: at 20-25°C: 14 days

at 2-8°C: 30 days

If the absorbance of working reagent is higher than 0.1 at 492 nm the reagent can not be used.

Assay conditions

Wavelength:	505 (480-520) nm
Temperature:	37°C
Cuvette:	1 cm light path
Read against:	reagent blank
Method:	endpoint (increasing)

Pipette into cuvette

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10 µl		
Standard		10 µl	
Sample			10 µl

Mix and read the absorbance (A) after a 5-minute incubation.

Calibration: (37°C, Cholesterol-oxidase method)

S1: Distilled water
S2: Cholesterol standard Cat. No.: 50611N or
Roche C.F.A.S. (Calibrator for automated system)
Randox Calibration Serum Level I or
Randox Calibration Serum Level II

Calibration frequency

Two point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{sample}}{A_{satndard}} \times C_{standard} = C_{sample}$$

A = Absorbance
C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Hitachi 717 analyzer (37°C).

Linearity

The test is linear up to 20 mmol/l (773mg/dl) cholesterol concentration.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.016 mmol/l (0,62mg/dl) Cholesterol concentration at 492 nm.

Precision

	Reproducibility		
	Average concentration	SD	CV%
Sample I.	3.63	0.025	0.67
Sample II.	4.62	0.056	1.22

	Repeatability		
	Average concentration	SD	CV%
Sample I.	4.78	0.070	1.46
Sample II.	7.14	0.059	0.82

Correlation

Comparative studies were done to compare our reagent with another commercial cholesterol assay on 66 human samples.
The results from these studies are detailed below.
Correlation coefficient: r=0.9975
Linear regression: y (mmol/l)= 1.011x-0.0164
(x= other commercial reagent, y= own reagent).

Specificity

Bilirubin 855 µmol/l (50mg/dl), lipid 450mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 0.57 mmol/l (10mg/dl) don't interfere with the assay up to the given levels.

NOTE

The circumstances of blood sampling influence the result.

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

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Richmond I. Clin. Chem. 19, 1350 (1973)
Face CF. Clin. Chem. 18, 901 (1982)