CHOLESTEROL KIT

(CHOD / PAP method)

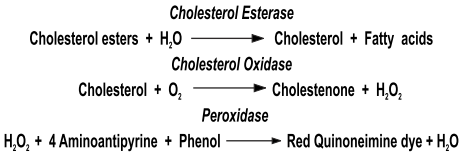
For the determination of Cholesterol in serum or plasma.
(For Invitro Diagnostic Use Only)

Summary

Cholesterol is the main lipid found in blood, bile and brain tissues. It is the main lipid associated with arteriosclerotic vascular diseases. It is required for the formation of steroids and cellular membranes. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Increased levels are found in hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephrotic syndrome, and cirrhosis. Decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anemias and liver diseases.

Principle

Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.



Normal reference values

Serum / Plasma (Suspicious) : 220 mg/dl and above
(Elevated) : 260 mg/dl and above

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

Contents	75 ml	2 x 75 ml	2 x 150 ml	2 x 250 ml
L1 : Enzyme Reagent 1	60 ml	2 x 60 ml	2 x 120 ml	2 x 200 ml
L2 : Enzyme Reagent 2	15 ml	2 x 15 ml	2 x 30 ml	2 x 50 ml
S : Cholesterol Standard (200 mg/dl)	5 ml	5 ml	5 ml	5 ml

Storage / stability

Contents are stable at 2-8^{0}C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : Pour the contents of 1 bottle of L2 (Enzyme Reagent 2) into 1 bottle of L1 (Enzyme Reagent 1).This working reagent is stable for at least 8 weeks when stored at 2-8^0 C. Upon storage the working reagent may develop a slight pink colour however this does not affect the performance of the reagent.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent 1) and 1 part of L2 (Enzyme Reagent 2). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum, EDTA plasma. Cholesterol is reported to be stable in the sample for 7 days when stored at 2-8^0 C. The sample should preferably be of 12 to 14 hours fasting.

Procedure

Wavelength / filter : 505 nm (Hg 546 nm) / Green
Temperature : 37^{0}C / R.T.
Light path : 1 cm

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

Addition Sequence	B (ml)	S (ml)	T (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Cholesterol Standard (S)	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37^{0}C for 5 min. or at R.T. (25^{0}C) for 15 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank, within 60 Min.

Calculations

$$\text{Cholesterol in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 200$$

Linearity

This procedure is linear upto 750 mg/dl. If the value exceeds this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Note

Anticoagulants such as flourides and oxalates result in false low values. The test is not influenced by Hb values upto 20 mg/dl and bilirubin upto 10 mg/dl.

References

Trinder, P., (1969) Ann. Clin. Biochem. 6 : 24
Allain, C.C., etal, (1974) Clin. Chem. 20 : 470
Flegg, H.M., (1972) Ann. Clin. Biochem. 10 : 79

System Parameters

Reaction	:	End Point
Wavelength	:	505 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	37^{0}C / R.T.
Incub. Time	:	5 min. / 15 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.01 ml
Reagent Vol.	:	1.00 ml
Standard	:	200 mg/dl
Factor	:	---
React. Slope	:	Increasing
Linearity	:	750 mg/dl
Units	:	mg/dl

CHO(2Rgt).02(P)

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