CHOLESTEROL

(CHOD / PAP method)

INTRODUCTION

Cholesterol is essential structural component of cell membranes and precursor of bile acids and all steroids hormones. This is why cholesterol has enormous significance for organism normal functioning. But there is also well established association between blood cholesterol concentration and coronary heart disease. Measurement of cholesterol serum level is valuable in prevention and monitoring cardiovascular disease. This determination is useful also for evaluation of intestine absorption, liver and gallbladder function.

METHOD PRINCIPLE

Enzymatic, colorimetric method with cholesterol esterase and cholesterol oxidase (CHOD/PAP).

$$\text{cholesteryl esters} + H_2O \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHE}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine dye} + 4H_2O$$

(Red coloured)

The colour intensity is proportional to the cholesterol concentration.

KIT CONTENTS

Reagent Name	Pack Size
R1 - Cholesterol Reagent	2 x 50 ml
R2 - Cholesterol standard	2 ml

Refer Standard vial mentioned the concentration of cholesterol standard.

WORKING REAGENT PREPARATION AND STABILITY

The reagent is ready to use.

The reagent when stored at 2-8°C is stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination.

CONCENTRATIONS IN THE TEST

Good's buffer (pH 6.4)	100 mmol/l
Phenol	5 mmol/l
4-aminoantipyrine	0.3 mmol/l
Cholesterol esterase (CHE)	> 3.2 µkat/l
Cholesterol oxidase (CHO)	> 1.67 µkat/l
Peroxidase (POD)	> 50 µkat/l
Phosphotungstic Acid	2.4 mmol/l
Magnesium Chloride	25 mg/dl

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents are usable when the absorbance of the working reagent is less than 0.150 (read against distilled water, wavelength λ=500 nm, cuvette l=1 cm, at temp. 25°C).
- The reagent and standards contain 0.09% sodium azide as a preservative. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 500 nm (Hg 546 nm)
- Thermostat at 37°C
- General laboratory equipment

SPECIMEN

Serum, EDTA or heparinized plasma (recommended: heparine lithium, sodium or ammonium salt) free from hemolysis.

Blood should be collected only if the patient has been fasting for minimum of 12 hours. Before blood collection patient should stay in rest position for about 30 minutes. Venous blood is recommended for cholesterol measurement.

Plasma cholesterol values have been reported to be 3% to 5% lower than serum cholesterol values.

Serum should be separated from red blood cells as soon as possible after blood collection.

Serum and plasma can be stored up to 3 days at 2-8°C or 6 months at -20°C. Nevertheless it is recommended to perform the assay with freshly collected samples.

PROCEDURE for CHOLESTEROL

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers. **Programme Sheets** are available on request.

Wavelength	500 nm
Temperature	20-25°C / 37°C
Cuvette	1 cm

Reagent	Blank (B)	Standard (S)	Test (T)
R1 Cholesterol Reagent	1000 µl	1000 µl	1000 µl
Bring up the temperature of determination. Then add,			
Distilled water	10 µl		
R2 - Cholesterol standard		10 µl	
Sample			10 µl

Mix well, incubate for 5 min. at 37°C or 10 min. at 20-25°C. Read the absorbance of the test A(T) and standard A(S) against reagent blank (RB).

CALCULATION

Cholesterol concentration = A(T) / A(S) x standard concentration

REFERENCE VALUES

Children < 4 Weeks	50 to 170 mg / dl
2 to 12 months	60 to 190 mg / dl
> 1 year	110 to 230 mg / dl
adults	< 200 mg / dl

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

- **Sensitivity / Limit of Quantitation:** 1.6 mg/dl (0.041 mmol/l)
- **Linearity:** up to 750 mg/dl (19.4 mmol/l)
- **Specificity / Interferences**

Haemoglobin up to 2.5 g/dl, ascorbate up to 62 mg/l, triglycerides up to 500 mg/dl and bilirubin up to 20 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements

LITERATURE

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SYSTEM PARAMETERS

Method	End Point
Wavelength	505 nm
Zero Setting	Reagent blank
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	5 mins
Delay Time	----
Read Time	----
No. of Reading	----
Interval Time	----
Sample Volume	0.01 ml (10 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer standard vial
Units	mg / dl
Factor	----
Reaction Slope	Increasing
Linearity	750 mg / dl

IVD

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