

Manual Procedure

Cat. No. 12841 R 2 x 50 ml
For 100 tests

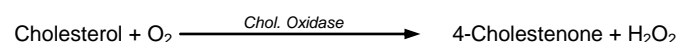
Cat. No. 12842 R 6 x 50 ml
For 300 tests

Cholesterol CHOD/PAP

Enzymatic colorimetric method

Liquid mono Reagent

Test principle



Dye = 4-(p-benzoquinone-monoimino)-phenazone

Serum cholesterol esters are hydrolyzed to cholesterol and free of fatty acids by cholesterol esterase enzyme. In the presence of oxygen and cholesterol oxidase, the cholesterol is converted to 4-cholestenone and hydrogen peroxide.

The oxidative condensation of phenol and 4-aminophenazone in the presence of peroxidases (POD) produces rose colour dye which is measured at 505 nm. The intensity of the colour produced is directly proportional to the cholesterol concentration of the sample.

Concentrations in the test

Reagent R		
PIPES = (Piperazine-1.4-bis (2-ethane-sulfonic acid)	8.6	mmol/L
Magnesium chloride	1.72	mmol/L
Phenol	5.3	mmol/L
4-Aminophenazone	0.29	mmol/L
POD (Peroxidase)	600	U/L
Cholesterol esterase	500	U/L
Cholesterol oxidase	500	U/L
Detergent, Stabilizer		
Standard : The Concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R: liquid mono reagent, ready to use.

The reagent is stable up to expiry date given on the label when stored at +2 → +8 °C. Stability after opening the bottle: 2 months at +2 → +8 °C.

Note: The reagents maybe go to slightly pink it is normal. Don't use if the reagents absorbance against distillate water more than 0.115.

Specimen collection and handling

Non-hemolyzed serum, heparinized, or EDTA plasma. Cholesterol is reported to be stable for 7 days at 2 - 8°C and for 6 months at - 20°C when properly protected against evaporation.

Calibrator / Standard

MediCal U Cat. No. 15011
Cholesterol STD. Cat. No. 16071

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 546 (500 - 560 nm)
Spectrophotometer	505 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator/ Standard	Sample
Distilled water	10 µl	--	--
Calibrator/ Standard	--	10 µl	--
Sample	--	--	10 µl
Reagent R	1000 µl	1000 µl	1000 µl

Mix, incubate for 5 min. at 37°C or 10 min. at 20 - 25 °C. Read absorbance (A). The color is stable for 30 min.

Calculation

$$\text{Conc. Cholesterol (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc. Cal./STD. (mg/dl)}$$

$$\text{mmol/L} \xleftrightarrow[0.0259 \times]{\times 38.7} \text{mg/dl}$$

Linearity

Up to 800 mg/dl (20.7 mmol/L).

If the result exceeds 800 mg/dl, repeat the test using diluted serum (1+4) with sodium chloride solution (0.9 %) and multiply the result by 5.

Interferences

1. Hemolysis: No significant interference of hemoglobin up to 500 mg/dl.
2. Ascorbic acid: No significant interference up to 100 mg/dl.
3. Conjugated bilirubin: No significant interference up to 20 mg/dl
4. Free bilirubin : As above.
5. A number of drugs and substances may affect cholesterol results.

Precaution

Reagents contain sodium azide. Don't swallow. Avoid any contact with skin and mucous membranes. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Reference range

Total Cholesterol CHOD - PAP	≤ 4 wk.	50 - 170	mg/dl
	2 - 12 mth.	60 - 190	mg/dl
	≥ 1 yr.	110 - 230	mg/dl
	Adults	< 200	mg/dl
HDL- Cholesterol	Adults	> 35	mg/dl
LDL- Cholesterol	Adults	< 155	mg/dl

References

1. Trinder, C. Clin. Chem. Clin. Biochem. 8 (1970) 658.
2. Weibhaar, D. Grossau, E. and All., Med. Welt 26 (1975) 387 - 390.
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4. Study group, European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: A policy statement of the European Atherosclerosis Society. European Heart Journal 1987, 8:77.
5. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AAC Press, Washington, D.C.