Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12211	R1	3 x 40	ml
For 180 tests	R2	3 x 20	ml
Cat. No. 12212	R1	3 x 70	ml
For 315 tests	R2	3 x 35	ml
Cat. No. 12213	R1	2 x 160	ml
For 480 tests	R2	2 x 80	ml

Cholesterol CHOD/PAP

Enzymatic colorimetric method

Liquid Reagents

Test principle

Cholesterol esters +$3H_2O$ —(*Chol. Esterase*)→ Cholesterol + 3 R-COOH

Cholesterol + O_2 —(*Chol. Oxidase*)→ 4-Cholestenone + H_2O_2

$2H_2O_2$ + 4-aminophenazone + ADPS —(*Peroxidase*)→ $4H_2O$+ Dye

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 ADPS and 4-aminophenazone in the presence of peroxides (POD) produces rose color dye which is measured at 550 nm.
The intensity of the color produced is directly proportional to the cholesterol concentration of the sample.

Concentrations in the test

Reagent R1		
PIPES = (Piperazine-1.4-bis (2-ethane-sulfonic acid)	100	mmol/L
Magnesium chloride	4.0	mmol/L
POD (Peroxidase)	2900	U/L
ADPS = (N-Ethyl-N- (3-sulfoproyl)-3-methoxyaniline)	0.65	mmol/L
Cholesterol esterase	500	U/L
Detergent, stabilizer		
Reagent R2		
4-Aminophenazone	1.0	mmol/L
Cholesterol oxidase	1000	U/L
Stabilizer		

Standard : The Concentration as indicated on vial.

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *liquid.*
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:
Mix 2 volumes of bottle R1 with 1 volume of bottle R2.
Stability : 4 weeks at 2 - 8 °C.

Note: Don't use if the reagent is turbid.

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 (520 - 560 nm)
Spectrophotometer	550 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
Working Reagent	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

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

$$\text{mmol/L} \underset{0.0259\ \times}{\overset{\times\ 38.7}{\rightleftarrows}} \text{mg/dl}$$

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

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