Manual
Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12240 R 1 x 50 ml
For 50 tests

Cat. No. 12241 R 2 x 50 ml
For 100 tests

LDL- Cholesterol

Precipitation method

Liquid Reagent

Test Principle

LDL- Cholesterol forms a precipitate by the action of the precipitating reagent heparin on the serum. The supernatant contains VLDL and HDL-Cholesterol which are measured by the use of the Cholesterol CHOD/ PAP method. LDL-Cholesterol is equal to the difference between total cholesterol and cholesterol in the supernatant.

Concentrations in the test

Precipitating Reagent R :		
Citrate buffer solution pH = 5.04	64.0	mmol/L
Heparin	≥ 50	KU/L
Standard : The Concentration as indicated on vial.		

Stability of reagent

Precipitating reagent R: *liquid, ready to use.*

The reagent is stable up to expiry date given on the label when stored
at +2 ➔ +8 °C.

Note: Don't use if crystals sediment appear in the reagent.

Additional requirement but not provided :
Cholesterol reagent Cat. No.12211,12212 or 12213.

Specimen collection and handling

1. Serum, heparinized or EDTA plasma.
2. Patient should be fasting 12 – 14 hours before the sample is taken.
3. LDL in serum is reported stable for 7 days at 2 - 8 °C, and for 3 months when frozen at - 20°C, and properly protected against evaporation.

Standard

LDL-Cholesterol STD. Cat. No. 16261

Quality control

Meditrol lipids N Cat. No. 15211
Meditrol lipids P Cat. No. 15221

Precipitation

Sample	100 µl
Precipitating Reagent	1000 µl
Mix, incubate for 10 min. at 20 - 25 °C. Centrifuge for 10 min. at a minimum of 4000 rpm.	
Separate supernatant within 1 hour and use it for test.	

Note: Turbid supernatant has to be diluted (1+1) with sodium chloride solution (0.9 %). Multiply result by 2.

Determination of LDL- Cholesterol

Please prepare the cholesterol working reagent as specified in the package insert of the cholesterol kits.

Procedure

Wavelength	Hg 546 (540 - 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	Standard	Sample
Distilled water	100 µl	--	--
Standard	--	100 µl	--
Supernatant	--	--	100 µl
Cholesterol working reagent	1000 µl	1000 µl	1000 µl
Mix. Incubate for 5 min. at 37°C or 10 min. at 20 - 25 °C. Read the absorbance (A) against reagent blank. The color is stable for 30 min.			

Calculation

$$\text{Cholesterol concentration in supernatant} = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times \text{Conc.}_{\text{Standard}}$$

LDL- Cholesterol (mg/dl) =

Total Cholesterol – (Cholesterol concentration in the supernatant x 11*)

Note: 11* is the dilution factor of sample with precipitant.

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

Linearity

Up to 300 mg/dl (7.77 mmol/L) .
If the result exceeds 300 mg/dl or supernatant is turbid repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

Triglycerides: No significant interference up to 400 mg/dl.

Precaution

Don't pipette by mouth.

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. Assmann, G. Internist, 20, (1979) , 559.
2. Lopez-virellu, M. F., Clin. Chem., 23, (1977) , 882.
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.