Manual
Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12220 R 1 x 50 ml
For 50 tests

Cat. No. 12221 R 2 x 50 ml
For 100 tests

HDL- Cholesterol

Precipitation method

Liquid Reagent

Test Principle

Chylomicrons, VLDL and LDL are precipitated quantitatively by adding phosphotungstic acid and magnesium ions to the sample.
After ccentrifugation, the cholesterol concentration in the high density lipoprotein (HDL), which remains in the supernatant, is measured using the Cholesterol CHOD/ PAP method.

HDL-Cholesterol esters + $3H^2O$ $\xrightarrow{Chol.Esterase}$ Cholesterol + 3 R-COOH

Cholesterol + O_2 $\xrightarrow{Chol.Oxidase}$ 4-cholestenone + H_2O_2

$2H_2O_2$ + 4 aminophenazone + ADPS $\xrightarrow{Peroxidase}$ 4H2O + 4-(p-benzoquinone- monoimino)-phenazone

ADPS = N-Ethyl-N- (3-sulfoproyl)-3-methoxyaniline

Concentrations in the test

Precipitating reagent R		
Phosphotungstic acid	0.55	mmol/L
Magnesium chloride	37.5	mmol/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.

Standard : the concentration is indicated on the vial.
Additional requirement but not provided :
Cholesterol reagent Cat. No.12211 or 12212 or 12213.

Note : Don't use the reagent if it has crystals or sediment.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma.
2. Serum must be separated from the blood clot as rapidly as possible.
3. Patient should be fasting 12 -14 hours before the sample is taken.
4. HDL-Cholesterol 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

HDL- Cholesterol STD. Cat. No. 16121

Quality control

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

Precipitation

Sample	500 µl
Precipitating Reagent	1000 µl
Mix, incubate for 10 min. at 20 - 25 °C. Centrifuge for 10 min. at a minimum of 4000 rpm or 2 min. at 12000 rpm.	
Separate supernatant within 2 hours and use it for cholesterol assay.	

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

Determination of HDL- Cholesterol

Prepare the cholesterol working reagent as specified in the package insert of the cholesterol kit.

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	1000µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37 °C or 10 min. at 20 – 25 °C. Read absorbance (A) against reagent blank. The color is stable for 30 min.			

Calculation

$$\text{HDL- Cholesterol (mg/dl)} = \frac{A_{Supernat}}{A_{Standard}} \text{ X Conc.}_{Standard} \text{ X } 3.0^*$$

Note: 3.0* is the dilution factor of sample with precipitant.

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

Linearity

Up to 150 mg/dl (3.88 mmol/L) .If the result exceeds 150 mg/dl or supernatant is turbid repeat the test by using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

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. Bilirubin: No significant interference up to 20 mg/dl.

Precautions

Avoid any contact of reagent with skin. Don't ingest.

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. Steele, B. W., Koehler, D.F., Azar, M., Blaskowski, T.P., Duba, K., Dempey, M.E.: Clin. Chem. 22(1976), 98.
2. Gordon, T. et al.: Am. J. Med., 62 (1977), 707.
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
4. Wiebe DA, Warnick GR. Measurement of high density lipoprotein cholesterol concentration. In: Rifai N, War-nick RG, Dominiczak H, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997, 12744.