Automated Procedure

Cat. No. 20250	R1	1	х	45	ml
	R2	1	х	15	ml
Cat. No. 20251	R1	2	х	45	ml
	R2	2	х	15	ml

Test Principle

When a sample is mixed with Reagent 1 the protecting reagent binds to LDL and protects LDL from enzyme reactions. Cholesterol esterase (CHE) and cholesterol oxidase (CO) react with non-LDL lipoproteins chylomicrons (CM), very low density lipoprotein (VLDL) and (HDL).

Hydrogen peroxide produced by the enzyme reactions with non-LDL cholesterol is decomposed by catalase in Reagent 1. When Reagent 2 is added, the protecting reagent is removed from LDL and catalase is inactivated by sodium azide (NaN₃). In this second process, CHE and CO react only with LDL-C. Hydrogen peroxide produced by the enzyme reactions with LDL-C yields a color complex upon oxidative condensation with N(2hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) and 4-aminoantipyrine (4AAP) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced, at approximately 600 nm, the LDL-C concentration in the sample can be calculated when compared with the absorbance of the LDL-C Calibrator.

Concentrations in the test

Reagent R1		
Buffer pH = 6.8	25.0	mmol/L
Cholesterol esterase	5000	U/L
Cholesterol oxidase	5000	U/L
H-DAOS	0.64	mmol/L
Catalase	1.0	mU/L
Detergent, stabilizer		
Reagent R2		
Buffer pH = 7.0	25.0	mmol/L
4-Aminophenazone	3.4	mmol/L
POD (Peroxidase)	20	KU/L
Stabilizer, preservative		

Stability of reagents

Reagent R1: liquid, ready to use.

Reagent R2: liquid, ready to use.

Use reagent R1 & R2 as supplied. Unopened bottles are stable up to expiry date given on the label when stored at $+2 \rightarrow +8$ °C. After opening the bottles, these solutions are stable for 30 days at 2-8 °C.

Notes:

- 1. Don't freeze reagents R1& R2.
- 2. Precipitation in the reagents is an indication of reagents instability.

Specimen collection and handling

- 1. Serum or heparinized 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.
- Store the specimen at 4 °C before analysis. For prolonged storage, specimens should be stored frozen at - 70 °C or lower.²
- Li Heparinate plasma values on average are recovered 3 % lower than serum concentrations. For EDTA plasma, ca. 9 % value decrease against serum is expected.

In vitro diagnostics

LDL-Cholesterol

Enzymatic selective protection method

MEDICHEM

Clinical Chemistry Reagents

Liquid Reagents

Calibrator

lipids HDL/LDL Calibrator Cat. No. 15871

Quality control

lipids level 1 Cat. No. 15211 lipids level 2 Cat. No. 15221

A quality control programme is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

Procedure



Linearity

Up to 400 mg/dl.

If the result exceeds 400 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

- Artificial lipid mixtures, as contained in some solutions for intravenous infusion (e.g. Intralipid®) can interfere with MEDICHEM LDL-C L-Type test principle. Samples from patients currently under such treatment are to be excluded from determination with MEDICHEM LDL-C L-Type reagent.
- Samples with triglyceride concentrations exceeding 1000 mg/dl should be diluted and reanalyzed. Dilute the sample with saline in appropriate relation and reanalyze. For obtaining the definite LDL result, multiply the concentration value of the diluted sample times the dilution factor.
- Ascorbic acid up to 50 mg/dl, free bilirubin up to 50 mg/dl, conjugated bilirubin up to 40 mg/dl, and hemoglobin up to 500 mg/dl do not interfere with the assay.

Precautions

- 1. The reagent is designed to be used on commercially available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications.
- 2. Don't use the Pretreatment reagent which was frozen by mistake.
- 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. Burtis, C. A, and Ashwood, E. P., Ed. Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, (1994).
- Rifai, N., Warnick, G. R. and Dominiczak, M. H., Ed. Handbook of Lipoprotein Testing. AACC Press, Washington, DC, USA, (1997). 2.
- Testing, AACC Press, Washington, DC, USA, (1997).
 Friedewald, W. T., Levy, R. I. and Frederickson, D. S. Estimation of the concentration of low density lipoprotein cholesterol in Plasma without use of the ultracentrifuge. Clin. Chem. 18, 449 502 (1972).
 The Expert Panel. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch. Intern. Med. 148, 36 69 (1988). 3.
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- 5. The Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Education, and Treatment of high Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA. 269, 3015 - 3023 (1993).

In vitro diagnostics