FUJIFILM

LDL-C L-Type

Read this instruction sheet thoroughly before use!

Enzymatic Selective Protection Method

For the quantitative determination of LDL-Cholesterol in serum and plasma

CE

419-24017-01
0124D9

Intended use

The LDL-C L-Type test is an *in vitro* assay for the quantitative determination of Low Density Lipoprotein Cholesterol (LDL-C) in serum and plasma.

Summary and explanation of the test

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, LDL-cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong positive relationship between LDL-C concentration and the incidence of CHD was reported[1]. Thus, there has been substantial interest in LDL-C measurements, and most clinical laboratories routinely perform LDL-C analysis. The currently accepted reference method is generally referred to as „beta quantification"[2], which involves ultracentrifugation. Because this method is labor intensive and technique dependent, it is not generally used for routine testing. The Friedewald formula[3] is most commonly used for routine purposes. However, since the formula estimates LDL-C from measurements of total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C), the LDL-C calculation depends on the accuracy and precision of the three measurements. The Wako LDL-C L-Type test is a homogeneous assay, which eliminates the preparatory steps or calculation, and thus, can be applied on automated analyzers.

Principle of the method

1st reaction (elimination of non-LDL cholesterol)

When a sample is mixed with Enzyme-Color Reagent (R1), polyanion and amphoteric surfactant protect 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 Enzyme-Color Reagent.

$$\text{CM-, VLDL-, HDL-} \rightarrow \text{Cholesterol} + H_2O + O_2$$

$$\xrightarrow[\text{CO}]{\text{CHE}} \Delta^4\text{ - Cholestenone} + \text{Fatty acids} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2\,H_2O$$

2nd reaction (color reaction of LDL cholesterol)

When Reacting Solution (R2) 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-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) and 4-aminoantipyrine (4-AA) 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.

$$\text{LDL-Cholesterol} + H_2O + O_2$$

$$\xrightarrow[\text{CO}]{\text{CHE}} \Delta^4\text{ - Cholestenone} + \text{Fatty acids} + H_2O_2$$

$$H_2O_2 + \text{4-AA} + \text{HDAOS} \xrightarrow{\text{POD}} \text{Blue color complex} + 3H_2O$$

Reagents

Contents and storage conditions

R1:	Enzyme-Color Reagent	Store at 2–10 °C
R2:	Reacting Solution	Store at 2–10 °C
R2 and R2		(Do not freeze)
R1:	Good's-Puffer, pH 6.8	25 mmol/L
Enzyme-Color Reagent	CHE (*from Pseudomonas*)	5,000 U/L
	CO (*from Nocardia*)	5,000 U/L
	HDAOS	0.64 mmol/L
	Katalase (*from bovine liver*)	1,000,000 U/l
	Ascorbat-Oxidase (*from Acremonium*)	5 U/mL
R2a:	Good's-Puffer, pH 7,0	25 mmol/L
Reacting Solution	4-AA	3.4 mmol/L
	POD (*from horseradish*)	20,000 U/L
	Sodium Azide (NaN_3)	0.095 %

Reagent preparation

R1: Use Reagent 1 as supplied. Unopened Reagent 1 is stable until expiration date at 2–10 °C. Opened Reagent 1 can be used for one month at 2–10 °C.

R2: Use Reagent 2 as supplied. Unopened Reagent 2 is stable until expiration date at 2–10 °C. Opened Reagent 2 can be used for one month at 2–10 °C.

CAL: Reconstitute one bottle of LDL-C Calibrator with 1 mL of distilled or deionized water. Keep the reconstituted LDL-C Calibrator in a refrigerator.

The reconstituted LDL-C Calibrator is stable for 7 days at 2–10 °C. The calibrator can be aliquoted and frozen once after reconstitution. Repeated freezing and thawing should be avoided.

Specimen collection and preservation

Use serum or heparin plasma as a specimen.

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.

Physical or chemical indication of instability

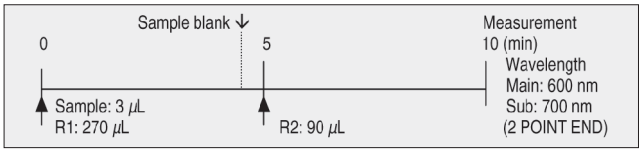
The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent's instability.

Instruments

The LDL-C reagent is designed to be used on commercially available automated analyzers such as Hitachi® 917 analyzer. Refer to the operating manual for a description of instrument operation and specifications.

Standard procedure

Temperature: 37 °C



This is the standard procedure. Instrument applications are available upon request.

Results

The final results are automatically calculated and printed in concentration units (mg/dL).

Warnings and precautions

- For *in vitro* diagnostic use.
- Only for professional use.
- Not to be used internally in humans and animals.
- Do not use reagents past the expiration date stated an each reagent container label.
- Do not use the reagents described above for any purpose other than described herein.
- R2 contains sodium azide (0.095 %) as a stabilizer. Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though this reagent contains minute quantities of sodium azide, drains should be flushed well with a large amount of water when discarding the solution.
- Clinical diagnosis must be determined with clinical symptoms and other test results by a physician.
- When using enzymatic methods for the determination of cholesterol esters, contamination and interference to other clinical chemistry assays on the same instrument in principle cannot be excluded. In the rare event of such a problem occurring, please refer to the instrument's manual for channel setting and washing procedure options.
- Artificial lipid mixtures, as contained in some solutions for intravenous infusion (e.g. Intralipid®) can interfere with Wako LDL-C L-Type test principle. Samples from patients currently under such treatment are to be excluded from determination with Wako LDL-C L-Type reagent.
- Patient's samples with a rare type of Hyperlipoproteinemia (Hyperlipoproteinemia Type III) can provide falsely results with LDL-C Type reagent[6].
- Samples with triglyceride concentrations exceeding 1,000 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 dilute sample times the dilution factor.
- When discarding the reagents, dispose of them according to local or national regulations.

Additional information (R1)

EUH208 Contains Mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one. May produce an allergic reaction.

Materials required but not supplied

LDL-C Calibrator
Quality control material
Automated analyzer

Calibrator

The values of Wako LDL-C Calibrator were assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL) and the calibrator value is around the medical decision level.

Quality control

A quality control program 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.

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Expected values

NCEP ATP's (USA) Decision Cut-off Points for LDL-C[4,5]

Desirable	< 130 mg/dL
Borderline High Risk for CHD	130–159 mg/dL
High Risk for CHD	160 mg/dL

Performance characteristics

Accuracy (Hitachi® 917)

The accuracy of this method was demonstrated by a recovery study.

Expected (mg/dL)	Observed (mg/dL)	Recovery (%)
15.0	15.6	104
30.6	31.2	102
76.2	76.1	100

Linearity

The linearity of Wako LDL-C L-Type test is 1–400 mg/dL. If the LDL-C value exceeds 400 mg/dL, dilute the sample with 1 part sample to 1 part saline, repeat the assay, and multiply the result by 2.

Comparison

Comparison studies were done to compare the Wako LDL-C L-Type assay with the reference method (beta-quantification) and a commercially available homogeneous LDL-C method. The results from these studies are detailed in the table below.

Method:	Wako LDL-C L-Type vs. Reference Method	Wako LDL-C L-Type vs. homogeneous LDL-C	
Specimen		Serum	Plasma
n=	60	60	60
MW (mg/dL)	$\bar{x}$ = 136.6; $\bar{y}$ = 137.1	$\bar{x}$ = 117.0; $\bar{y}$ = 119.3	$\bar{x}$ = 109.1; $\bar{y}$ = 110.8
Regression analysis (mg/dL)	y = 0.97x + 5.12	y = 1.018x + 0.135	y = 0.98x + 4.18
Correlation coefficient	r = 0.983	r = 0.986	r = 0.988

Precision (Hitachi® 917)

Within-run precision

Sample #	Replicates	Mean (mg/dL)	SD	CV (%)
1	10	101.2	0.62	0.61
2	10	164.5	0.71	0.43

Total precision

Three levels of controls were run in duplicate and in duplicate runs for a period of 24 days. The data was collected according to NCCLS EP5-T2 Guideline.

No. of assay days	Mean (mg/dL)	SD	CV (%)	S_{wr}	S_T
24	126.2	0.761	0.60	0.751	1.54
24	225.8	1.229	0.54	1.570	2.77

Sensitivity

The minimum detectable level of this method is estimated to be 1 mg/dL.

Specificity (Hitachi® 917)

Ascorbic acid, free bilirubin, conjugated bilirubin, and hemoglobin did not interfere with the assay at levels up to 50, 50, 40, and 500 mg/dL, respectively.

Ascorbic Acid (mg/dL)	None	10	20	30	40	50
LDL-C (mg/dL)	129.8	129.0	129.3	129.4	128.2	128.3

Free Bilirubin (mg/dL)	None	10	20	30	40	50
LDL-C (mg/dL)	103.0	103.6	102.6	102.7	102.6	102.1

Conjug. Bilirubin (mg/dL)	None	8	16	24	32	40
LDL-C (mg/dL)	108.7	108.5	108.5	107.5	106.3	106.3

Hemoglobin (mg/dL)	None	100	200	300	400	500
LDL-C (mg/dL)	124.9	125.1	125.1	124.6	124.8	124.8

References

1. Rifai N, Bachorik PS, and Albers JJ. Lipids, Lipoproteins, and Apolipoproteins. In: Burtis CA, Ashwood ER (eds). Tietz Fundamentals of Clinical Chemistry, 5th Edition, W.B. Saunders Company, Philadelphia, 2001: PART IV, 24; 463, 480.
2. Recommandation of the Second Joint Task Force of European and other Societies on Coronary prevention. Prevention of coronary heart disease in clinical practice; Eur Heart J 1998; 19: 1434-1503.
   Rifai, N., Warnick, G. R. and Dominiczak, M. H., Ed. Handbook of Lipoprotein Testing. AACC Press, Washington, DC, USA, (1997).
3. Bachorik PS. Measurement of low-density lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997. p. 145-160.
   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 preparative ultracentrifuge. Clin. Chem. 18:499-502 (1972).
4. Schaefer EJ, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Wamick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997. p. 25-48.
   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).
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, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA. 269:3015-3023 (1993).
6. Esteban-Salán M, Guimón-Bardesi A, de la Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Río E, Analytical and Clinical Evaluation of Two Homogeneous Assays for LDL-Cholesterol in Hyperlipidemic Patients. Clin Chem 2000; 46(8), 1121-1131.

Ordering information

Code No.	Product	Package
419-24017	LDL-C L-Type R1	R1: 4 x 60 mL
419-24027	LDL-C L-Type R2	R2: 4 x 20 mL
991-24015	LDL-C L-Type R1	R1: 1 x 500 mL
991-24025	LDL-C L-Type R2	R2: 1 x 500 mL
419-24032	LDL-C Calibrator	CAL: 4 x for 1 mL

Manufacturer:

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