FUJIFILM

Code No. 993-00404
999-00504

L-Type LDL-C

Intended use

The L-Type LDL-C is an in vitro assay for the quantitative determination of low density lipoprotein cholesterol (LDL-C) in serum or plasma.

Summary and explanation of the test

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In addition to total cholesterol, LDL-C is 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 of the 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 L-Type LDL-C is a homogeneous assay, which eliminates the preparatory steps or calculation, and thus, can be applied on automated analyzers.

Principle of the method

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 lipoprotein (chylomicron (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 (NaN_3). 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 oxidase condensation with N-(2-hydroxy-3-sulfo-propyl)-3,5-dimethoxyaniline (HDAOS) and 4-aminoantipyrine (4AA) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced, at approximately 600nm, the LDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C / LDL-C Calibrator.

Reagents

(1) Reagent 1

25 mmol/L Good's buffer, pH 6.8 containing CHE (5,000 U/L from *Pseudomonas*), CO (5,000 U/L from *Nocardia*), HDAOS (0.64 mmol/L), catalase (1,000,000 U/L from bovine liver).
Store at 2-10℃.

(2) Reagent 2

25 mmol/L Good's buffer, pH 7.0 containing 4AA (3.4 mmol/L), POD (20,000 U/L from horseradish), and NaN_3 (0.095%).
Store at 2-10℃.

Warnings and precautions

For in vitro diagnostic use.
Not to be used internally in humans and animals.
Do not use reagents past the expiration date stated on each reagent container label.
Do not use the reagents described above for any purpose other than described herein.
Reagent 2 contains sodium azide as a preservative. 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.

Physical or chemical indications 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 instability.

Instruments

The 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.

Specimen collection and preparation

Use serum or plasma as a specimen. Store the specimen at 4℃ before analysis. Specimens should be stored frozen at −70℃ or lower[1].

Warning/Biohazard

It is recommended that specimen collection be carried out in accordance with CLSI Document M29-A3. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Procedure for Hitachi® 917

Materials supplied

Refer to the section entitled ''Reagents''.

Materials required but not supplied

Hitachi® 917 analyzer
HDL-C / LDL-C Calibrator.
Quality control material
All analyzer applications should be validated in accordance with NCEP and CLIA recommendations. For further assistance call Wako Diagnostics Technical Service Department at 1-877-714-1924.

Reagent preparation

Reagent 1: Use Reagent 1 as supplied. Unopened Reagent 1 is stable until expiration date at 2-10℃. Opened Reagent 1 can be used for one month at 2-10℃.

Reagent 2: Use Reagent 2 as supplied. Unopened Reagent 2 is stable until expiration date at 2-10℃. Opened Reagent 2 can be used for one month at 2-10℃.

Calibrator : Reconstitute one bottle of HDL-C / LDL-C Calibrator with 3 mL of distilled or deionized water. The reconstituted HDL-C / LDL-C Calibrator is stable for 7 days at 2-10℃.

Test procedure

Parameter setting (Hitachi® 917)

Temperature: 37℃

Reagent	L-Type LDL-C
ANALYZE	
CH TEST/TYPE	LDL-C/SERUM
ASSAY	2POINT END-10
POINT	16-34-0-0
WAVELENGTH (SUB/MAIN)	700/600
SAMPLE VOL (NORMAL)	2.4-0.0-0
(DEC.)	
(INC.)	
DILUENT	( )-0
REAGENT VOL R1	210-0-( )-0
R2	0-0-( )-0
R3	70-0-( )-0
R4	0-0-( )-0
ABS LIMIT	
PROZONE LIMIT	−32000 0 LOWER
CELL DET.	DET. 1
CALIBRATION	
CALIB TYPE	LINEAR
POINT	2/2
WEIGHT	0
AUTO CALIBRATION	0
SD LIMIT	999.9
DUPLICATE LIMIT	500
SENSITIVITY LIMIT	0
S1 ABS LIMIT	−32000/32000
RANGE	
TEST#	
UNIT	mg/dL
REPORT NAME	
DATA CODE	
CONTROL INTERVAL	
INST. FACTOR	a=1.0 b=0.0
TECHNICAL LIMIT	
EXPECTED VALUES	
STD CONC	CONC.POS.VOL.PREDIL.
1	0 H2O 2.4 0.0
2	*1 *2 2.4 0.0
3	0 0 0 0.0
4	0 0 0 0.0
5	0 0 0 0.0
6	0 0 0 0.0
K-FACTOR	

*1 Input the assigned value of the calibrator.

*2 Input the position of the calibrator.

Results

The final results are automatically calculated and printed in concentration.

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both 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.

Calibrator

The values of HDL-C / 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.

Linearity

The linearity of L-Type LDL-C 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.

Expected values

NCEP ATP's 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

Comparison

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

	Wako LDL vs. Reference Method	Wako LDL vs. Homogeneous LDL	
		Serum	Plasma
n	60	60	60
Mean (md/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	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.

Number of assay days	Mean (mg/dL)	SD	CV (%)	Swr	ST
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

Conjugated 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. Burtis, C. A. and Ashwood, E. R., Ed. Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994.
2. Rifai, N., Warnick, G. R. and Dominiczak, M. H., Ed. Handbook of Lipoprotein Testing. AACC Press, Washington, DC, USA, 1997.
3. 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).
4. 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).

Ordering information

Code No.	Product	Package
993-00404	L-Type LDL-C Reagent 1	2 × 60 mL
999-00504	L-Type LDL-C Reagent 2	2 × 20 mL
990-28011	HDL-C/LDL-C Calibrator	4 × for 3 mL

Revision Date ; Jun. 1, 2018

Manufactured by
FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome, Chuo-Ku Osaka 540-8605, Japan
Tel : +81-6-6203-3749
Fax : +81-6-6203-1917
www.wako-chem.co.jp

Distributed by
FUJIFILM Wako Diagnostics U.S.A. Corporation

1025 Terra Bella Ave., Mountain View, CA 94043 U.S.A.
Tel : 877-714-1924
Fax : 804-271-0449
www.wakodiagnostics.com

FUJIFILM Wako Chemicals Europe GmbH

Fuggerstr. 12, 41468 Neuss, Germany
Telephone : +49 2131 311 272
Fax : +49 2131 311 110
www.wako-chemicals.de

18.06.13K06