Code No. 991-00101
997-00201
997-72591
993-72691

L-Type HDL-C

Intended use

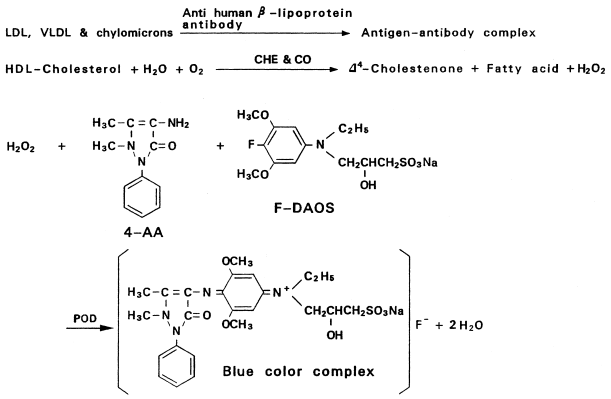
The L-Type HDL-C is an in vitro assay for the quantitative determination of high density lipoprotein cholesterol (HDL-C) in serum.

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, HDL-C has become an important tool used to assess an individual risk of developing CHD since a strong negative relationship between HDL-C concentration and the incidence of CHD was reported.[1] Thus, there has been substantial interest in HDL-C measurements, and most clinical laboratories routinely perform HDL-C analysis. Selective chemical precipitation techniques are widely used for the determination of HDL-C such as heparin-manganese, dextran-magnesium, and phosphotungstate-magnesium.[2] However, these techniques require physical separation via centrifugation, which is not suited to large scale lab use. L-Type HDL-C eliminates the precipitation procedure by employing a specific antibody, and thus, can be applied on automated analyzers.

Principle of the method

Anti human β-lipoprotein antibody in Reagent 1 binds to lipoproteins (LDL, VLDL, and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when Reagent 2 is added. Cholesterol esterase (CHE) and cholesterol oxidase (CO) in Reagent 2 react only with HDL-C. Hydrogen peroxide produced by the enzyme reactions with HDL-C yields a blue color complex upon oxidase condensation with FDAOS [N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt] and 4-aminoantipyrine (4AA) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced at apporoximately 600 nm, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C/LDL-C Calibrator.



Reagents

(1) Reagent 1
30 mmol/L Good's buffer, pH 7.0 containing 4AA (0.9 mmol/L), POD (2400 U/L from horseradish), and anti human β-lipoprotein antibody, sheep.
Store at 2-10℃. Do not freeze.

(2) Reagent 2
30 mmol/L Good's buffer, pH 7.0 containing CHE (4000 U/L from Pseudomonas), CO (20000 U/L from Nocardia), and FDAOS (0.8 mmol/L).
Store at 2-10℃. Do not freeze.

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.
UDI (Unique Device Identification) is on the box. Keep box until reagent is finished.

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 instrument's operating manual for a description of instrument operation, specifications, channel setting, and washing procedure options to avoid assay contamination.

Specimen collection and preparation

Use serum as a specimen. Sample should be drawn from a fasting patient. Store the specimen at 4℃ before analysis. HDL-C is stable for 4 days at 4-6 ℃. Specimens frozen at −20℃ show statistically but not clinically significant decreases in HDL-C measured at 7-14 days.[2]
Triglyceride levels up to 1,200 mg/dL will not effect the results of the HDL-C assay. If a specimen has triglyceride concentrations that exceed 1,200 mg/dL, the sample should be diluted with 1 part sample and 2 parts saline, repeat the assay and multiply the result by 3.
Refer to the work of Young for drug effects on serum HDL-C levels.[4]

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 contact Wako Diagnostics Technical Service Department at 1-877-714-1924 or e-mail diagnostics@wakousa.com.

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 : Accurately add 3 mL of distilled or deionized water to dissolve the contents of each calibrator. 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 HDL-C
ANALYZE	
CH TEST/TYPE	HDL-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	0
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 a 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 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. Quality Control materials are intended for use only for monitors of accuracy and precision. The NCEP Lipid Standardization Program recommends two levels of controls; one in the normal range (35-65 mg/dL) and one in the abnormal range (<35 mg/dL) for decision making.

Calibrator

The values of HDL-C/LDL-C Calibrator were assigned by procedures traceable to the Centers for Disease Control (CDC) reference methods and the calibrator value is around the medical decision level.

Linearity

The linearity of L-Type HDL-C is 1-180 mg/dL. If the HDL-C value exceeds 180 mg/dL, dilute the sample with 1 part sample to 2 parts saline, repeat the assay, and multiply the result by 3.

Expected values

REFERENCE VALUES FOR HIGH-DENSITY LIPOPROTEIN CHOLESTEROL (mg/dL)

	Males								Females							
Age in	Percentiles							Age in	Percentiles							
Years	5	10	25	50	75	90	95	Years	5	10	25	50	75	90	95
5-9	38	43	49	55	64	70	75	5-9	36	38	48	52	60	67	73
10-14	37	40	46	55	61	71	74	10-14	37	40	45	52	58	64	70
15-19	30	34	39	46	52	59	63	15-19	35	38	43	51	61	68	74
20-24	30	32	38	45	51	57	63	20-24	33	37	44	51	62	72	79
25-29	31	32	37	44	50	58	63	25-29	37	39	47	55	63	74	83
30-34	28	32	38	45	52	59	63	30-34	36	40	46	55	64	73	77
35-39	29	31	36	43	49	58	62	35-39	34	38	44	53	64	75	82
40-44	27	31	36	43	51	60	67	40-44	34	39	48	56	65	79	88
45-49	30	33	38	45	52	60	64	45-49	34	41	47	58	68	82	87
50-54	28	31	36	44	51	58	63	50-54	37	41	50	62	71	84	92
55-59	28	31	38	46	55	64	71	55-59	37	41	50	60	73	85	91
60-64	30	34	41	49	61	69	74	60-64	38	44	51	61	75	87	92
65-69	30	33	39	49	52	74	75	65-69	35	38	49	62	73	85	96
70+	31	33	40	48	56	70	75	70+	33	38	45	60	71	82	92

According to the National Cholesterol Education Program (NCEP) guidelines, a level of 35 mg/dL is the recommended cut-off point and the level less than 35 mg/dL is considered a risk factor for coronary artery disease (CAD).

Burtis, C.A. and Ashwood, E.R., Ed. Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994.

The second report of the NCEP ATP (ATPII), released in June 1993, identified a high HDL-cholesterol (≧60 mg/dL) as a ''negative'' risk factor, one that reduces CHD risk.

The Expert Panel. 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 1993; 269: 3015-23.

Performance characteristics

Accuracy (Hitachi® 917)
The accuracy of this method was demonstrated by a recovery study.

Expected (mg/dL)	Observed (mg/dL)	Recovery (%)
31.3	30.2	96.5
14.8	15.3	103.4
7.5	7.6	101.3

Precision (Hitachi® 917)
Within-run precision

Sample #	Replicates	Mean (mg/dL)	SD	CV (%)
1	20	31.4	0.36	1.15
2	20	53.6	0.32	0.60
3	20	73.1	0.70	0.96

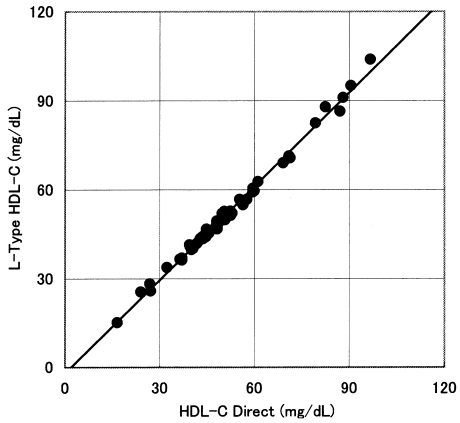
Total precision
Three levels of controls were run in duplicate and in duplicate runs for a period of 20 days. The data was collected according to NCCLS EP5-T2 Guideline.

Number of assay days	Mean (mg/dL)	SD	CV (%)	Swr	ST
22	68.9	1.67	2.42	0.54	2.03
22	23.2	0.56	2.41	0.16	0.59
22	112.2	2.38	2.12	0.94	2.80

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

Correlation Data (Hitachi® 917)
HDL Cholesterol

	x	y		x	y		x	y		x	y
1	16.5	15.2	16	42.6	43.0	31	48.5	48.7	46	61.1	62.8
2	24.0	25.6	17	42.8	43.2	32	49.8	52.1	47	69.1	69.1
3	26.8	28.3	18	42.9	43.6	33	50.1	50.9	48	70.9	71.4
4	27.1	25.9	19	43.4	44.1	34	50.5	52.8	49	71.3	70.8
5	32.3	33.8	20	43.8	43.6	35	50.7	50.0	50	79.3	82.5
6	36.4	36.6	21	44.1	44.6	36	52.4	52.9	51	82.4	87.9
7	37.0	37.0	22	44.7	44.2	37	52.4	52.4	52	87.0	86.5
8	37.0	36.3	23	44.8	46.8	38	52.5	51.4	53	88.0	91.1
9	39.4	41.5	24	45.5	45.5	39	52.9	52.2	54	90.5	95.1
10	39.7	40.7	25	45.6	45.4	40	55.3	56.8	55	96.7	104.0
11	40.1	39.9	26	45.9	46.4	41	56.4	55.0			
12	40.1	39.9	27	48.1	48.4	42	57.6	56.8			
13	40.1	40.7	28	48.1	49.5	43	59.5	59.3			
14	40.5	40.2	29	48.2	46.9	44	59.5	60.5			
15	41.7	41.9	30	48.3	48.3	45	59.9	59.7			



n = 55
y = 1.052x − 1.982
r = 0.997

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
HDL-C (mg/dL)	51.8	51.4	51.5	51.0	51.7	51.6

Free bilirubin (mg/dL)	NONE	10	20	30	40	50
HDL-C (mg/dL)	50.1	50.2	50.2	50.4	50.5	51.1

Conjugated bilirubin (mg/dL)	NONE	8	16	24	32	40
HDL-C (mg/dL)	53.0	52.4	52.3	51.9	51.3	51.1

Hemoglobin (mg/dL)	NONE	100	200	300	400	500
HDL-C (mg/dL)	53.2	52.9	52.7	52.6	52.2	52.3

References

1. Rifai, N. and Warnick, G. R., Ed. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins. AACC Press, Washington, DC, USA, 1994.
2. Burtis, C. A, and Ashwood, E. R., Ed. Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994.
3. 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).
4. Young, Donald S., M. D., Ph. D. Effects of Drugs on Clinical Laboratory Tests. AACC Press, Fourth Edition, Washington, DC, 1995.

Ordering information

Code No.	Product	Package
991-00101	L-Type HDL-C Reagent 1	4 × 600 mL
997-00201	L-Type HDL-C Reagent 2	4 × 600 mL
997-72591	L-Type HDL-C Reagent 1	4 × 270 mL
993-72691	L-Type HDL-C Reagent 2	4 × 90 mL
990-28011	HDL-C/LDL-C Calibrator	4 × for 3 mL

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