LDL Cholesterol D

Enzymatic colorimetric. Liquid

Quantitative determination of LDL cholesterol

CE IVD

PACKAGING

Ref.: 101-0535	Cont.: 1 x 30 / 1 x 10 mL
Ref.: 101-0516	Cont.: 1 x 60 / 1 x 20 mL

Store at 2 - 8º C

CLINICAL SIGNIFICANCE

The LDLc particle is lipoproteins that transport cholesterol to the cells. Often called "bad cholesterol" because high levels are risk factor for coronary heart disease and are associated with obesity, diabetes and nephrosis [1,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Direct determination of serum LDLc (low-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation steps.

The assay takes place in two steps.

– 1º Elimination of lipoprotein no-LDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

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

– 2º Measurement of LDLc

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{TOOS} + \text{4-AA} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the LDLc concentration in the sample.

REAGENTS

R 1 Enzymes	PIPES	50 mmol/L
	Cholesterol esterase (CHE)	≥ 600 U/L
	Cholesterol oxidase (CHOD)	≥ 500 U/L
	Catalase	≥ 600 KU/L
	TOOS	2 mmol/L
R 2 Enzymes	PIPES	50 mmol/L
	4 – Aminoantipyrine (4-AA)	4 mmol/L
	Peroxidase (POD)	≥ 4 KU/L
HDLc/LDLc CAL	Standard. Lyophilized human serum	

PRECAUTIONS

HDLc/LDLc CAL: Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

TRACEABILITY: Values are assigned according to the requirements of the Method Evaluation Protocol for Manufacturers" of the US National Reference System, CRMLN.

PREPARATION

- **R 1 and R 2**: Are ready to use.
- **HDLc/LDLc CAL:** Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C and contaminations are prevented during their use.

- **R 1 and R 2**: Once opened is stable 4 weeks at 2 - 8º C.
- **HDLc/LDLc CAL**: Once reconstitute 30 hours at 20-25º C, 2 weeks at 2-8º C or 3 months –20º C.Do not use reagents over the expiration date.

Signs of reagent deterioration:

Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 600 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum [1]: After sampling, the test should be performed without delay. Repeated freezing and thawing should be avoided.

Stability of the sample: 7 days at 2 - 8º C .

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 600 (590 - 700) nm
   Cuvette: . . . . . 1 cm. light path
   Temperature . . . . . .37º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R 1 (µL)	300	300	300
Standard (µL)	--	4	--
Sample (µL)	--	--	4

4. Mix and incubate for 5 min. at 37º C.
5. Add:

R 2 (µL)	100	100	100

6. Mix and incubate for 5 min. at 37º C.
7. Read the absorbance (A), against the Blank.

CALCULATIONS

$$\frac{(A)Sample - (A)Blank}{(A)Standard - (A)Blank} \text{ x Standard.conc.} = \text{mg/dL of LDLc in the sample}$$

Conversion factor: mg/dL x 0.02586 = mmol/L

1 g/L = 100 mg/dL

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1,5,6]

Optimal	< 100 mg/dL
Near or above optimal	100 - 129 mg/dL
Borderline high	130 - 160 mg/dL
High	> 160 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 10 mg/dL to *linearity limit* of 976 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L and multiply the result by 2.

Precision:

	Intraserie (n= 20)		Interserie (n= 20)	
Media (mg/dL)	31.4	67.8	32.1	68.1
SD	0.42	1.11	0.92	2.02
CV (%)	1.35	1.64	2.87	2.97

Sensibility: 1 mg/dL = 0.001784 (A).

Accuracy[10,11]: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient $(r)^2$: 0.99123.

Regression equation: y= 0.914x + 1.58283.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

The assay is unaffected by icteric samples. No interferences were observed with ascorbic acid up to 50 mg/dL, hemoglobin up to 0,5 g/dL, bilirubin up to 30 mg/dL, rheumatoid factors up to 1000 IU/mL or lipemic samples up to 1200 mg/dL. Lipemic samples with a triglyceride concentration >1200 mg/dL should be diluted 1/10 with NaCl 9 g/L and multiply the result by 10.

BIBLIOGRAPHY

1. Naito H. K., et al, Clin Chem, 41: 132-133, 1995.
2. Seidel d., et al, Internist, 28: 606-314, 1987.
3. Weiland H. and Seidel D., J Lip Res, 24: 904-909, 1983.
4. Friedewald w.F., et al, Clin Chem, 18:499-502, 1972.
5. Clinical Laboratory Diagnostics: use and Assesment of Clinical Laboratory Results: First Edition T-H Books Germany; p 172.
6. Rifai N., et al, Clin Chem, 38 : 150-160, 1992.
7. National Cholesterol Education Program. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA, Vol.285, No. 19; p.2846-2897 Publication 2001.
8. Armstrong V., et al, Arztl Lab, 31: 325-330, 1985.
9. Bachorik P.S. and Ross J.W., Clin Chem, 41: 1414-1420, 1995.
10. Passing H. and Bablok W., J Clin Chem Clin Biochem, 21: 709-720, 1983.
11. Bablok W., et al, J Clin Chem Clin Biochem, 26: 783-790, 1988.

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