Audit Diagnostics

CE

LDLc -D

LDL Cholesterol D

Enzymatic colorimetric. Liquid

Quantitative determination of LDL cholesterol
IVD

Store at 2-8ºC

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

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

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

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

– 2º Measurement of LDLc

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

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

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

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

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.

REAGENTS

R 1 Enzymes	PIPES pH 7.0 (20ºC) Cholesterol esterase (CHE) Cholesterol oxidase (CHOD) Catalase N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS)	50 mmol/L ≥600 U/L ≥500 U/L ≥600 KU/L 2 mmol/L
R 2 Enzymes	PIPER pH 7.0 4 – Aminoantipyrine (4-AA) Peroxidase (POD)	50 mmol/L 4 mmol/L ≥ 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 2-8ºC and contaminations are prevented during their use.
- **R 1 and R 2**: Once opened is stable 4 weeks at 2-8ºC.
- **HDLc/LDLc CAL**: Once reconstitute 2 weeks at 2-8ºC or 3 months –20ºC.
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 2-8ºC .

PROCEDURE
1. Assay conditions:
   Wavelength: . . . . . 600 (590-700) nm
   Cuvette: . . . . . .1 cm. light path
   Temperature: 37ºC
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 37ºC.
5. Add:

R 2 (µL)	100	100	100

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

CALCULATIONS

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

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: LIPID CONTROL
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 7 mg/dL to *linearity limit* of 1000 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)	71,75	108,6	177,6	98	153	207
SD	0,44	1,05	1,93	2,44	3,39	3,63
CV (%)	0,62	0,96	1,09	2,5	2,21	1,75

Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 54 samples were the following:
Correlation coefficient (r): 0,99.
Regression equation: y= 0,9634x + 5,35.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
No interferences were observed with ascorbic acid up to 50 mg/dL, hemoglobin up to 500 mg/d, bilirubin up to 30 mg/dL, rheumatoid factors up to 1000 IU/mL or lipaemic samples up to 1200 mg/dL.Lipaemic samples with a triglyceride concentration >1200 mg/dL should be diluted 1/10 with NaCl 9 g/L and multiply the result by 10.

NOTES
Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Kaplan A et al. Lipoprotein. Clin Chem The C.V. Mosby Co. St Louis.
2. Okada M. et al. Low-density lipoprotein cholesterol can be chemically measured J. Lab. Clin. Mad., 1998; 132, 195-201.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

Manufactured By: AUDIT DIAGNOSTICS
Business & Technology Park, Carrigtwohill, Co. Cork (Ireland)
Tel: 00353 - (0) 21 – 4533 652 *Fax:* 00353 - (0) 21 – 4533 653
E-mail: info@auditdiagnostics.ie *Website:* www.auditdiagnostics.ie