Atlas Medical

LDL CHOLESTEROL
Direct Enzymatic Colorimetric Method
For In-Vitro Diagnostic
Store at 2-8°C

INTENDED USE

For the measurement of cholesterol concentration in human serum.

Principle

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.

1. Elimination of lipoprotein no –LDL:

Cholesterol esters $\xrightarrow{CHE}$ cholesterol+ fatty acids

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

$2H_2O_2$ $\xrightarrow{catalase}$ $2H_2O + O_2$

2. Measurement of LDLc

Cholesterol esters $\xrightarrow{CHE}$ cholesterol+ fatty acids

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

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

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.

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

Precautions

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.

Reagents

R1 Enzymes	• PIPES Ph 7.0 (20°C)	50mmol/L
	• Cholesterol esterase (CHE)	≥600U/L
	• Cholesterol oxidase (CHOD)	≥500 U/L
	• Catalase	≥600 KU/L
	• N-Ethyl-N-(2-hydroxy-3-sulfopropyl) -3-methylaniline(TOOS)	2mmol/L
R2 Enzymes	• PIPER Ph 7.0	50mmol/L
	• 4-Aminoantipyrine (4-AA)	4mmol/L
	• Peroxidase (POD)	≥4KU/L
LDL CAL.	standard	

Preparation

- R1 and R2 : are ready to use.
- Calibrator:
  1. Reconstitute with 1.0 ml of distilled water mix well , making sure there is no material left on the walls.
  2. Let stand the vial for at least 2 hours before using.
  3. Mix well before using.

Additional equipment

- spectrophotometer or colorimeter measuring at 600±10nm
- matched cuvettes 1.0 cm light path.
- General laboratory equipment.

Storage and stability

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use.
- Signs of reagent deterioration: Presence of particles and turbidity.
- R1 AND R2 : Once opened is stable 4 weeks at 2-8 °C.
- LDLc cal:once reconstitute 2 weeks at 2-8 °C or 3 months -20 °C.

Samples

- Serum, After sampling, the test should be performed without delay. Repeated freezing and thawing should be avoided.
- Stability of the samples :7 days at 2-8°C

Procedure

1. Assay conditions
   Wavelength……600 ±10 nm
   cuvette……1cm. light path
   Temperature……37°C
2. Adjust the instrument to zero with distelled water.
3. Pipette into a cuvette

	Blank	Standard	Sample
R1(µL)	300	300	300
Standard(µL)	--	4	--
Sample(µL)	--	--	4

4. Mix and incubate for 5 minutes at 37°C.
5. Add:

R2(µL)	100	100	100

6. Mix and incubate for 5 minutes at 37°C.
7. Read the absorbance (A) , against the blank.

Calculations

$$\frac{\text{(A) Sample}}{\text{(A) standard}} \times \text{standard concentration}$$

=mg/dL of LDLc in the sample

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

Quality control

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

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

Reference values

RISK	LDL-Cholesterol level
Optimal	< 100 mg/dl (2.59 mmol/l)
Near optimal	100-129 mg/dl (2.59-3.34 mmol/l)
Borderline	130-159mg/dl (3.37-4.12mmol/l)
High	160-189mg/dl (4.14-4.89 mmol/l)
Very high	≥ 190mg/dl (4.92 mmol/l)

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 ½ with NaCl 9 g/L and multiply the result by 2 .

Precision:

	Intra-assay (n=20)			**Intra-assay (n=20)**		
Mean (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 ATLAS 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/dl , bilirubin up to 30 mg/dl, rheumatoid factors up to 1000 IU/ml of 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 .

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