DIRECT LDL CHOLESTEROL

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QUANTITATIVE DETERMINATION OF LDL CHOLESTEROL

PRINCIPLE

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

The assay takes place in two steps.

Elimination of lipoprotein non-LDL Cholesterol.

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

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

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

Measurement of LDL Cholesterol

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

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

$$2H_2O_2 + \text{TOOS} + \text{4-AA} \xrightarrow{\text{POD}} 2H_2O + O_2$$

The Intensity of the color formed is proportional to the LDL Cholesterol concentration in the sample.

CLINICAL SIGNIFICANCE

The LDL Cholesterol particles are 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 with clinical and other laboratory data.

REAGENT COMPONENT AND CONCENTRATION

R1	GOOD pH 7.0 (2^{0}C)	50 mmol/L
	Cholesterol esterase (CHE)	380 U/L
	Cholesterol oxidase (CHOD)	380 U/L
	Catalase	400 U/mL
	N-(2hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (TOOS)	0.45 mmol/L
R2 Enzymes	GOOD pH 7.0	50 mmol/L
	4-Amino antipyrine (4-AA)	1.00 mmol/L
	Peroxidase (POD)	100 µ/L
HDLc/LDLc CAL	Standard, Lyophilized human serum	

PRESENTATION:

Store all reagents at 2-8^{0}C		No. of Bottles		
		20ml/ 50 T	40ml/100 T	80ml/200T
1	1LDL Cholesterol	1	1	1
2	2 LDL Cholesterol	1	1	1
3	HDL/LDL Calibrator	1	1	1
4	Distilled Water	1	1	1

WORKING REAGENT PREPARATION.

Reagents are ready to use as supplied.

CE

An ISO 13485: 2012 & GMP Certified Company

HDL/LDL Calibrator Preparation & Stability:

Refer the calibrator insert before use.

REAGENT 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^{0}C and contamination are prevented during their use.

R1 and R2: Once opened is stable 4 weeks at 2-8^{0}C.

PRECAUTION:

Do not use reagents over the expiration date.

Sign of reagent deterioration.

- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 546 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SPECIMEN COLLECTION

Serum : After Serum Separation, the test should be performed without delay.

Repeated freezing and thawing should be avoided.

Stability of the sample : 7 days at 2-8^{0}C.

PROCEDURE FOR SEMI & FULLY AUTOMATED ANALYZERS.

REACTION PARAMETERS:

- Type of Reaction : End Point
- Wavelength : 546 nm
- Flow cell temperature : 37^{0}C
- Incubation : 5 + 5 min at 37^{0}C
- Sample Volume : 5 µl
- Reagent Volume (R1+R2) : 375 + 125 µl
- Calibrator Concentration : As mentioned on vial
- Light Path : 1 cm
- Zero setting with : Distilled water

PROCEDURE:

Pipette into Test Tube	Blank	Standard	Sample
R1 (µL)	375	375	375
Standard (µL)	-	5	-
Sample (µL)	-	-	5

Mix and Incubate for 5 min at 37^{0}C.

Add:

R2 (µL)	125	125	125

Mix and Incubate for 5 min 37^{0}C.

Read the absorbance (A), against the Blank.

CALCULATIONS

$$\text{LDL Cholesterol (mg/dl)} = \frac{\text{A Sample}}{\text{A Calibrator}} \times \text{Calibrator value}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

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 tolerance.

EXPECTED VALUE

Level of the risk

Desirable	<100 mg/dL
Medium	130-160mg/dL
High	> 160mg/dL

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

PERFORMANCE CHARACTERISTICS

Measuring range : From detection limit of 3.7 mg/dL to linearity limit of 1000 mg/dL.

Precision:

	Intra –assay		
Mean (mg/dL)	32.9	50.8	101.4
SD	0.3	0.2	0.7
CV	0.8	0.5	0.7

Inter – assay		
32.8	50.0	100.0
0.4	0.7	1.1
1.3	1.5	1.1

INTERFERENCES

No Interferences were observed with ascorbic acid up to 50 mg/dL, hemoglobin up to 500 mg/dL or bilirubin up to 30 mg/dL.
A list of drugs and other interfering substances with LDL cholesterol determination has been reported by young et al [8.4]

LINEARITY

The method is linear upto a concentration of 1000 mg/dl. Specimens with LDL values above 1000 mg/dL should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.

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