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LDL CHOLESTEROL

Diagnostic reagent for the in-vitro quantitative determination of LDL Cholesterol in human serum on both manual and automated systems.

REF:BS.1/LC05.005.0025	50 test	REF:BS.1/LC10.005.0050	100 test

CLINICAL SIGNIFICANCE

Cholesterol and triglycerides, being nonpolar lipid substances (insoluble in water), need to be transported in the plasma associated with various lipoprotein particles. Plasma lipoproteins are separated by hydrated density; electrophretic mobility; size; and their relative content of cholesterol, triglycerides, and protein into five major classes: chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).
The LDL particle 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.

METHOD PRINCIPLE

Direct determination of serum LDL-c (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

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-one} + H_2O_2$$

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

2- Measurement of LDLc

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4-en-3-one} + H_2O_2$$

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

REAGENT COMPOSITION

R 1: Enzyme - GOOD 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 - 1200 U/L - 2 mmol/L
R2: Reagent 2 - GOOD pH 7.0 - 4 – Aminoantipyrine (4 -AA) - Peroxidase (POD)	 - 50 mmol/L - 4.0 mmol/L - 5000 U/L
HDLc/LDLc Calibrator	Standard Lyophilized human serum

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the LDL Cholesterol reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Bioscien LDL Cholesterol reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8˚C. Once opened.

- R 1 and R 2: Are ready to use. Once opened is stable 4 weeks at 2 -8ºC
- HDLc/LDLc CALIBRATOR: Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents. Once reconstitute 1 week at 2 - 8ºC or 5 weeks - 20ºC.

Deterioration
The ***Bioscien*** LDL Cholesterol reagent is normally clear. Do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION

Serum, 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.

SYSTEM PARAMETERS

Wavelength	600 nm (590 – 700 nm)
Optical path	1 cm
Assay type	End-point
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:100 400 µl 4 µl
Temperature	37 ˚C
Incubation time	5 min. at 37˚C
Zero adjustment	Reagent Blank
Sensitivity	3.7 mg/dl
Linearity	1000 mg/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	*Standard*	*Specimen*
Reagent (R1)	300 µl	300 µl	300 µl
Standard (Calibrator)		4 µl	
Specimen			4 µl
Mix and incubate for 5 minutes at 37°C			
Reagent (R2)	100 µl	100 µl	100 µl

Mix and incubate for 5 minutes at 37°C. Measure absorbance of specimen "A" and standard "A" against reagent blank.

CALCULATION

LDL Cholesterol conc. (mg/dl) = $\frac{\text{(A specimen)}}{\text{(A standard)}}$ x standard conc.

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

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	90	90	90	90
Mean mg/dl	63.2	107	65.2	112
SD.	0.64	1.89	0.3	0.7
CV. %	1.01	1.76	0.45	0.60

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

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** LDL Cholesterol reagent compared with commercial reagent of the same methodology performed on 90 human sera give a correlation of 0.998.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 3.7 mg/dl.

Linearity
The reaction is linear up to LDL Cholesterol concentration of 1000 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Haemolysis
No significant interference from haemoglobin up to 500 mg/dl.

Icterus
No significant interference from bilirubin up to 30 mg/dl.

Others
No interferences were observed with ascorbic acid up to 50 mg/dl
A list of drugs and other interfering substances with LDL cholesterol determination has been reported by Young et. al.

EXPECTED VALUES

Serum	***mg/dl***
Desirable	<100
Medium	130-160
High	>160

DYNAMIC RANGE

3.7 - 1000 mg/dl

REFERENCES

1. Ellefson RD and Caraway WT: Fundamentals of clinical chemistry. Ed Tietz NW 1976; p506.
2. Kaplan A Et al. Lipoprotein. Clin Chem The C.V. Mosby Co. St Louis.
3. Okada M. et al. Low -density lipoprotein cholesterol can be chemically measured J. Lab. Clin. Mad., 1998; 132, 195 -201.
4. Trinder, P, Ann. Clin. Biochem. 1969; 6: 24.
5. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
6. Young DS. Effe cts of disease on Clinical Lab. Tests, 4th ed AACC 2001.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

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CE